

USER GUIDE

Echo® Dose-Response Application

Version 1.7

MARCH 2018

FOR RESEARCH USE ONLY



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Documentation for Echo® Dose-Response Application, Version 1.7

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United States: 6,416,164; 6,548,308; 6,603,118; 6,612,686; 6,642,061; 6,666,541; 6,707,038; 6,710,335; 6,746,104; 6,802,593; 6,808,934; 6,809,315; 6,849,423; 6,855,925; 6,869,551; 6,893,836; 6,893,115; 6,916,083; 6,932,097; 6,938,987; 6,938,995; 6,991,917; 7,070,260; 7,090,333; 7,185,969; 7,270,986; 7,354,141; 7,405,072; 7,405,395; 7,439,048; 7,454,958; 7,481,511; 7,717,544; 7,899,645; 7,900,505; 7,901,039; 8,107,319; 8,389,295; 8,503,266; 8,770,69; 9,212,250; 9,586,215; 9,908,133.

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Additional patents are approved and pending in the United States and other countries.

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CHAPTER 1 | Preface

This section provides information about who the intended audience is, the software requirements, technical support resources, and documentation conventions used. It includes the following sections.

- [About this Guide](#)
- [Intended Audience](#)
- [Software Requirements](#)
- [Technical Support Resources](#)
- [Related Documentation](#)
- [Documentation Conventions](#)

About this Guide

This guide describes the key features of the Echo® Dose-Response application.

Intended Audience

The intended audience for this guide is laboratory personnel who have been trained to use Labcyte products. Researchers can use the Echo Dose-Response application to create transfer protocols for the Echo Liquid Handler that map transfers of samples and reagents to assay plates for direct dilution.

Software Requirements

To use the Echo® Dose-Response Application, the following items are required:

- Echo Liquid Handler instrument using server version 2.4.x or later and Echo.Net Framework version 1.7.
- Software license key from Labcyte Inc.
- Computer system meeting the following requirements:
 - **Operating system:** Microsoft® Windows® 7 (32-bit mode or 64-bit mode), and Microsoft® Windows 10 (64-bit mode) which must be run in Windows 7 Compatibility Mode; Windows .Net Framework 4.6



Note: Echo® Dose-Response Application will run in 32-bit mode on a Windows 64-bit Operating System.

- **CPU:** Intel® Core™ i5 or later
- **Memory:** 4 GB or greater
- **Network connection:** 10/100/1000 BaseT
- **Network protocol:** TCP/IP
- **Hard drive:** 4 GB Free Space Available
- **Video:** 1280 x 1024 resolution

The Echo® Dose-Response Application works closely with the Echo client software and should be installed on the same client PC.

The Echo Software Compatibility Matrix table below documents the versions of software supported by this guide and their compatibility.

Table 1: Echo Software Version Compatibility Matrix

Echo Client Software	Echo Application Software	Tempo Automation Control Software	Echo.Net Framework
2.4.5 - 2.5.x	1.5.4 or later	1.5.2 or later	1.5.4 or later
2.4.5 - 2.6	1.6.x	1.6.x	1.6.x
2.4 - 2.6	1.7	1.7* and 2.0*	1.7
3.0	1.7	2.0*	1.7



Note: For information on the compatibility of a specific software version, please contact your local Labcyte representative.

* Check with your Labcyte Sales Representative for availability.

Technical Support Resources

For technical support issues, support requests can be submitted via email to support@labcyte.com.

For telephone support, call (877) 742-6548.

Related Documentation

Labcyte documentation consists of the following publications:

- *Echo Dose-Response Quick Start Guide*
- *Echo Plate Reformat User Guide*
- *Echo Plate Reformat Quick Start Guide*
- *Echo Cherry Pick User Guide*
- *Echo Cherry Pick Quick Start Guide*
- *Echo Array Maker User Guide*
- *Echo Array Maker Quick Start Guide*
- *Echo Plate Audit User Guide*
- *Echo Plate Audit Quick Start Guide*
- *Echo Combination Screen User Guide*
- *Echo Combination Screen Quick Start Guide*
- *Echo 21CFR11 Compliance Manager User Guide*
- *Echo 21CFR11 Compliance Manager Quick Start Guide*
- *Echo 21CFR11 Compliance Manager Admin User Guide*
- *Echo 21CFR11 Compliance Manager Admin Quick Start Guide*
- *Echo 500 Series Liquid Handler User Guide*
- *Echo 525 Series Liquid Handler User Guide*
- *Echo 65XT Series Liquid Handler User Guide*
- *Echo Software Applications Integration Guide*
- *Echo Liquid Handler Software Integration Guide*
- *Tempo Automation Control Software User Guide*
- *Access Single Robot and Dual Robot Hardware Guide*

Documentation Conventions

Style	Purpose
<i>blue italicized text</i> (PDF, Web only)	Cross references, link, Web addresses
courier std	Commands, filenames, directories, paths, user input
bold text	Interactive interface objects, keys, buttons
<i>italicized text</i>	Book titles, glossary terms

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CHAPTER 2 | Getting Started

This chapter describes the basic tasks to start, set up, and exit the Echo Dose-Response application. It includes the following topics.

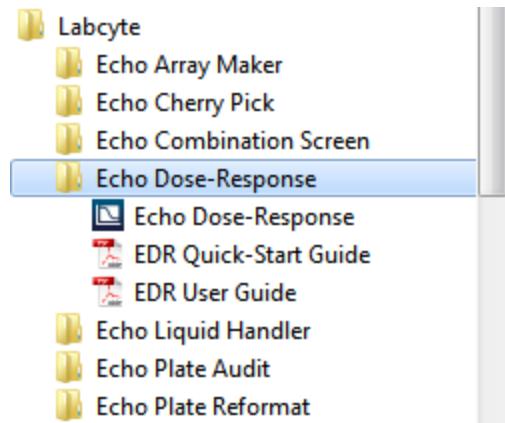
- [Starting the Software](#)
- [Activating the Instrument](#)
- [Understanding the Main Window](#)
- [Setting Up the Software](#)
- [Shutting Down the Software](#)

Starting the Software

To start the Echo Dose-Response application:

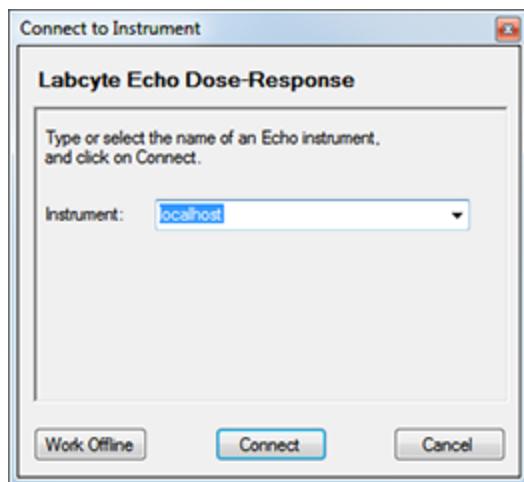
1. Open the **Start** menu.
2. Select **All Programs (or Programs)** > **Labcyte** > **Echo Dose-Response**.
3. Select the **Echo Dose-Response** application.

Figure 1: Location of Echo Dose-Response application



Note: To create a shortcut to the **Echo Dose-Response** application on the computer desktop, right-click on the **Echo Dose-Response** icon and select **Send to** and **Desktop**.

After launching the application, the **Connect to Instrument** prompt is displayed.

Figure 2: Connect to instrument dialog box

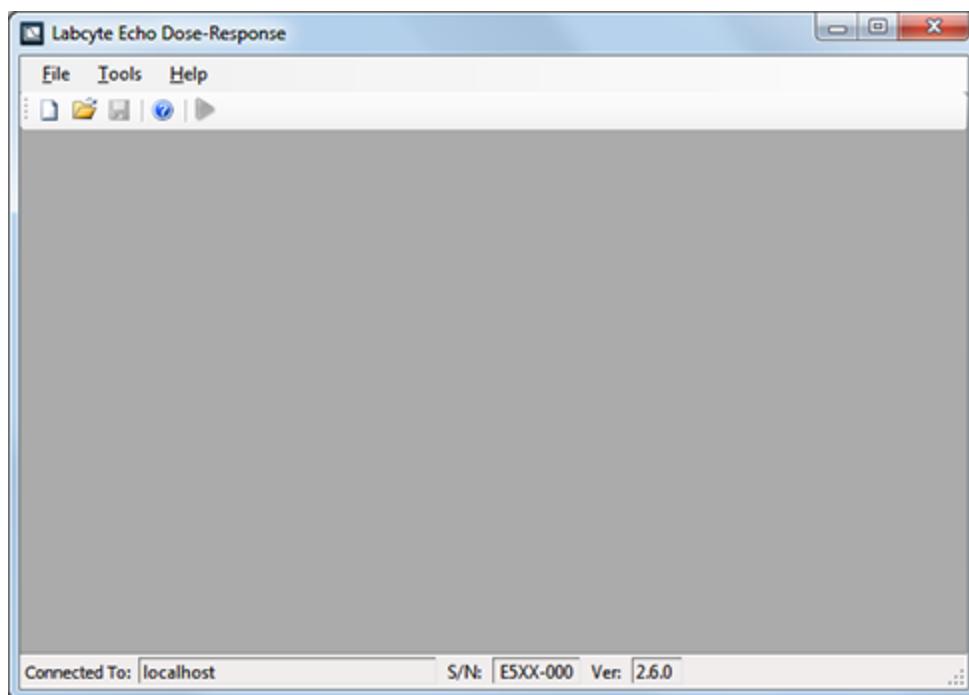
The **Connect to Instrument** dialog box connects the Echo Dose-Response application to a specific Echo instrument. The user can select an instrument from the dropdown list or type in the instrument name or IP address. If there are no instruments listed for **Instrument**, it means that the application has never connected to an instrument. After the first time a user connects to the instrument from the application, that Echo instrument will be added to the dropdown list in the application.



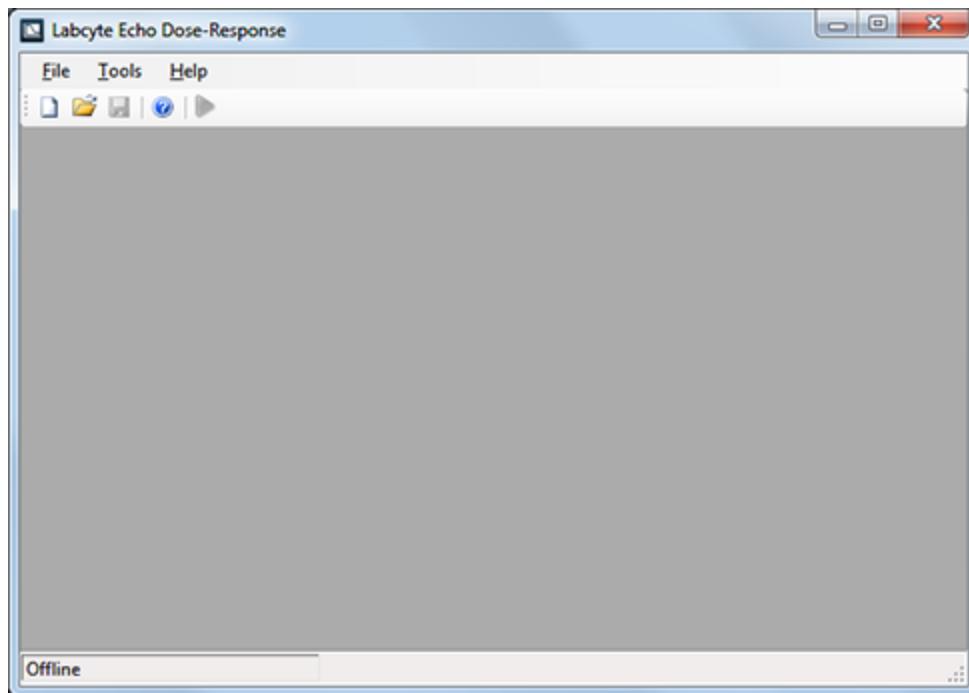
Note: Activating an instrument allows the user to run an actual protocol on a licensed Echo instrument. The user can still connect to the Echo instrument and create a protocol even if the licensing requirements have not been completed, but must click the **Work Offline** button to work offline. The protocol can be created but cannot be run until the licensing requirements have been met. If the user recently installed or upgraded the software, the instrument will need to be activated. See [Activating the Instrument](#) for more information.

4. Choose one of the following start options:

- **Connect:** Select an Echo Liquid Handler from the **Instrument** menu and click **Connect**. The Main Window opens with the connection status and instrument information at the bottom of the screen. This option enables the user to execute the sample or reagent transfer as soon as the transfer protocol is ready.

Figure 3: Echo Dose-Response application connected to an Echo Liquid Handler

- **Work Offline:** Click **Work Offline**. The Echo Dose-Response application opens with the connection status set to *offline* at the bottom of the screen. This option enables the user to create a transfer protocol and run a simulation without connecting to the Echo Liquid Handler.

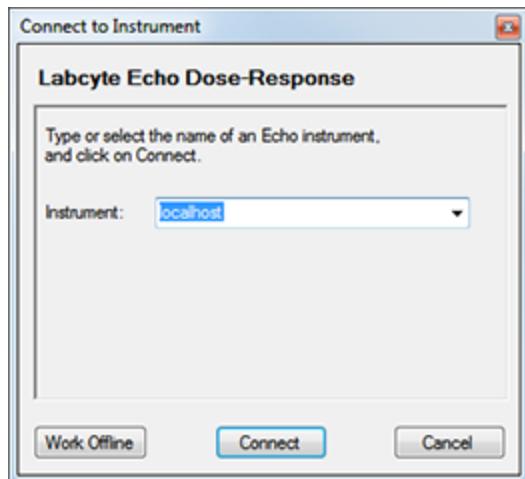
Figure 4: Echo Dose-Response application offline

Activating the Instrument

To run a protocol created from the Echo Dose-Response application on an Echo Liquid Handler, the user must activate this application for each Echo Liquid Handler. Each application has to be activated for the instrument connected.

- The Echo Dose-Response application must be activated with the software license key provided by Labcyte.
 - If the Echo Dose-Response application was installed with the Echo Liquid Handler by a Labcyte support engineer, the activation step should not be necessary.
1. Start the Echo Dose-Response application. See [Starting the Software](#) for more information.
 2. In the **Connect to Instrument** dialog box, type the name or IP address of the Echo server to connect to and click **Connect**.

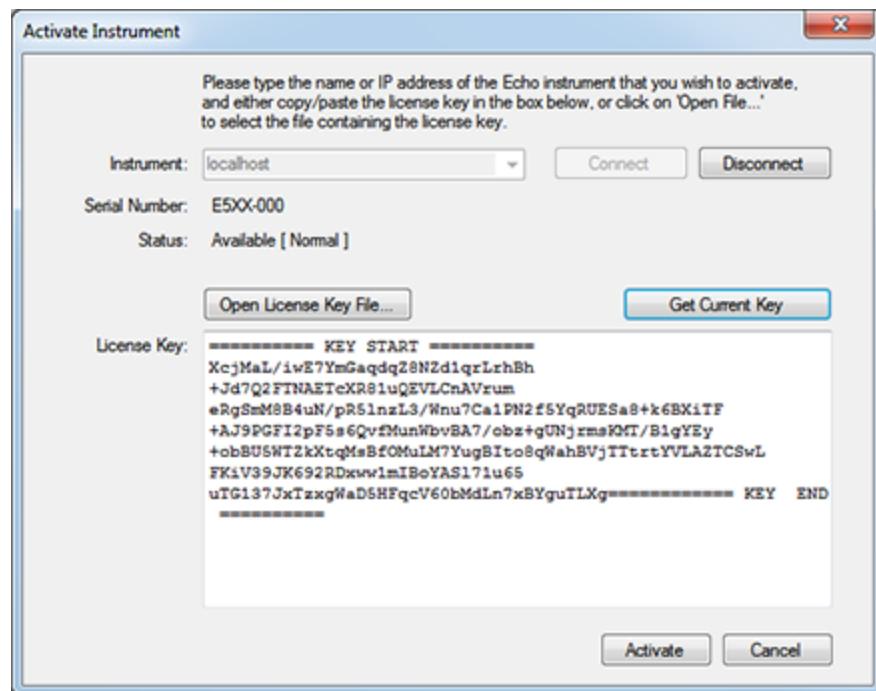
Figure 5: Connect to instrument dialog box



3. Click the Tools tab in the **Toolbar** and select the **Activate Instrument** option.
4. Enter the license key. The license key is an encrypted block of text that authorizes the user to run the Echo Dose-Response application with a specific Echo Liquid Handler.

The license key can be entered in either of the following ways:

- Copy the license key from the file received from Labcyte and paste it into the **License Key** text box.
- Click the **Open License Key File** button and browse for the license key file.

Figure 6: Activate instrument screen

Note: The **Get Current Key** function is a useful troubleshooting tool used to verify that the key is written correctly to the instrument. When the **Get Current Key** button is clicked, the application searches for the license key and inserts it into the **License Key** text box. In case of an error, send the encrypted key to Labcyte to determine if the license key is corrupted or if it applies to a different instrument.

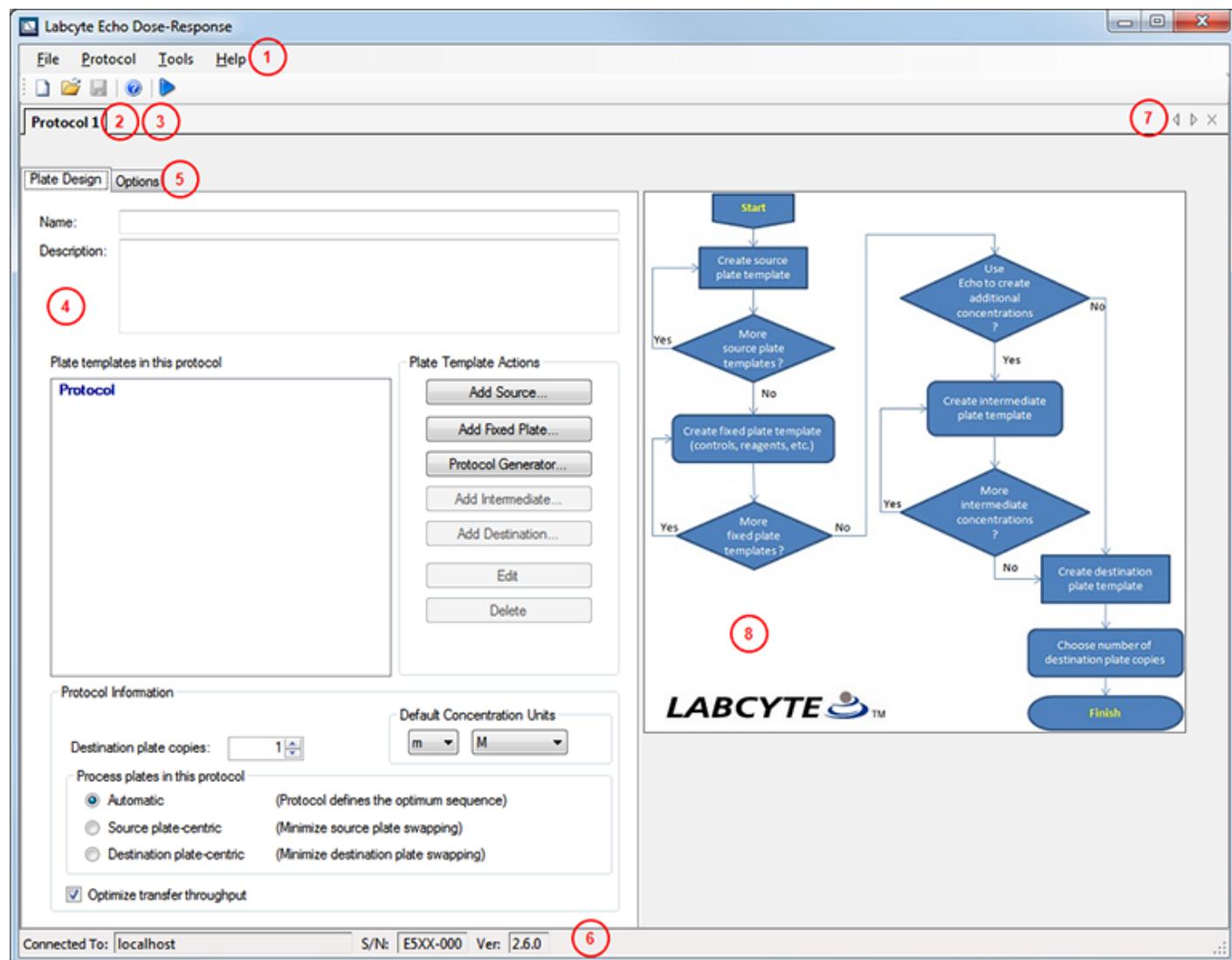
5. Click the **Activate** button.

The Echo Liquid Handler should be activated now. This procedure is not required again unless a different instrument is activated or the application needs to be upgraded.

Understanding the Main Window

When a new or existing protocol is opened, the **main Window** displays the **Protocol** window.

Figure 7: Main Window



The table below describes the buttons/fields or sections in the **Main Window** and their functionality.

Table 2: Callout table for Main Window

Callout Number	Name	Description
1	Toolbar	The toolbar contains software menus that provide options to open and save transfer protocols, set up the software, define Labware, run the protocol, customize protocol options, and display the online Help. The icons in the toolbar provide shortcuts to the most frequently selected options, such as the Run Protocol function. For a detailed description, see Toolbar .
2	Protocol window	The protocol window is labeled with the protocol file name. The software uses an .edr file extension and stores the file in the default directory: C:\Labcyte\Echo\Protocols. The default directory can be changed to a user specified directory.
3	Protocol window tabs	The window tabs open the following software windows: <ul style="list-style-type: none"> • Main Protocol window • Options Window
4	Main Protocol window	The main Protocol window is the first window that is displayed when a new or existing protocol is opened. It contains all of the protocol data necessary to perform the transfer. For a detailed description, see Protocol Tab .
5	Options window	The Options window displays protocol-specific options, such as survey history, output file type, and report formats. For a detailed description, see Protocol Options Tab .
6	Status bar	The status bar displays Echo connection information, such as instrument name, serial number, and the version of Echo client software that is running.
7	Navigation and Exit buttons	The left and right arrows become active when there are too many protocols open to view all of the file names. The arrows shift the protocol tabs left or right to display the file names. The Exit icon closes the displayed protocol.
8	Workflow and Template preview	The workflow diagram is displayed whenever a protocol is open. It serves as a reference tool for developing a dose-response protocol. When the user selects any template in the template tree (left pane), the workflow diagram is replaced with a graphical representation of the selected template.

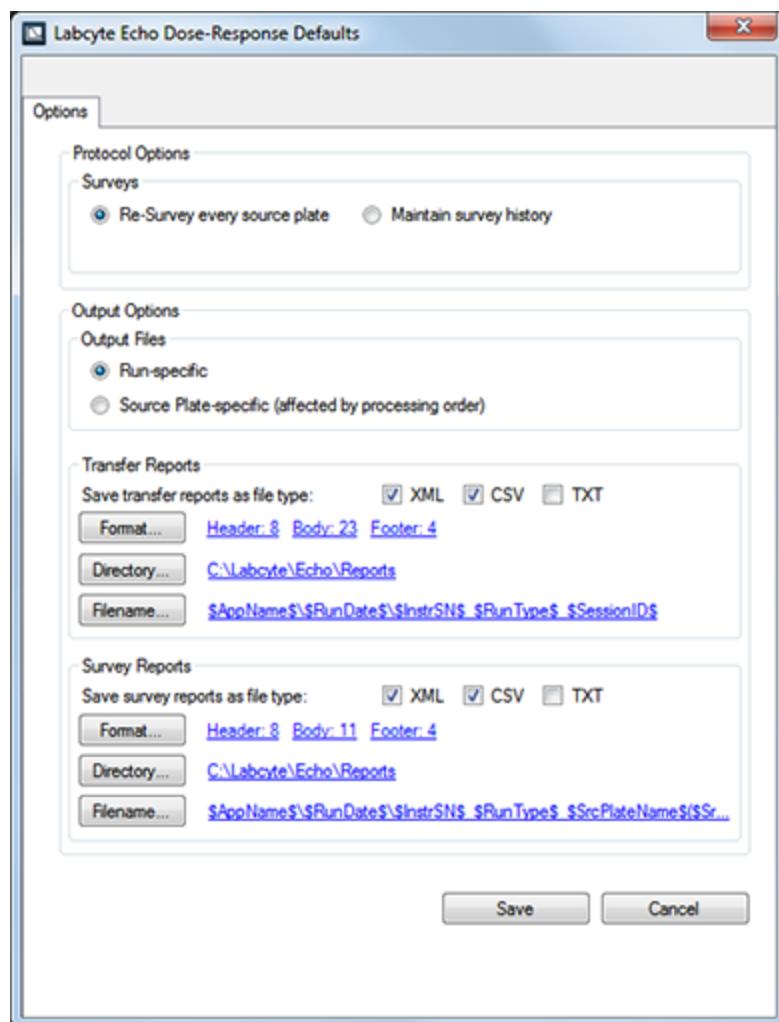
Setting Up the Software

The Echo Dose-Response application is simple to set up. Default settings, such as output file type and report format, can be modified here. The Echo Dose-Response application provides survey data in three file formats (csv, xml, and txt) with reporting options that can be customized to meet protocol-specific needs.

To change the default settings, and set up preferences:

1. Open the **Tools** menu and select **Preferences**.

Figure 8: Preferences/Options dialog box



2. Select **Maintain survey history** to re-use the current plate survey for more than one transfer operation for a specified period of time. For more information, see [Surveys](#).
3. Select **Run-specific** or **Plate-specific** for the output files.
 - **Run-specific** — Stores all of the source/destination transfer results in one file. The protocol data is not included.
 - **Plate-specific** — Stores all of the source/destination transfer results in separate files for each plate.
4. Customize the transfer and survey reports:
 - **File type** — Select XML, CSV, and/or TXT file formats.
 - **Format** — Customize the transfer/survey report header, body, and footer.
 - **Directory** — Set or change the directory location for the transfer/survey report.
 - **Filename** — Create or change the filename template for the transfer/survey report.

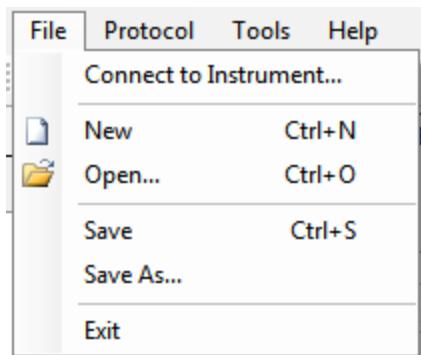
These options are used as default settings for all transfer protocols. To change the options for an individual protocol, use the Options tab. For more information, see [Protocol Options Tab](#).

Shutting Down the Software

There are two ways to exit the Echo Dose-Response application:

- Open the **File** menu and select **Exit**.

Figure 9: Exit function in the File menu



- Click the **Close** icon in the application.

Figure 10: Close window icon



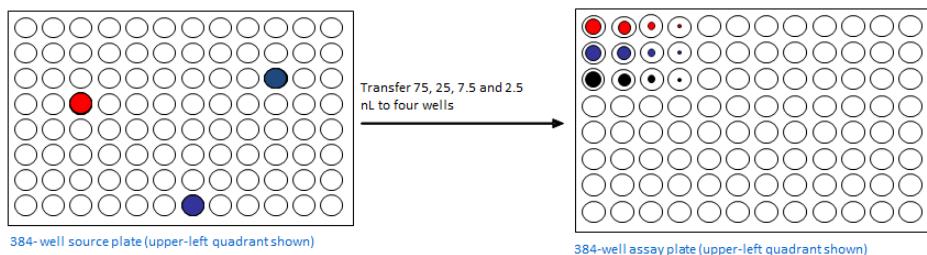
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CHAPTER 3 | Introduction

The Echo® Dose-Response application develops a protocol that instructs the Echo Liquid Handler to perform one or more liquid transfer steps. The application is compatible with all Echo Liquid Handlers. Protocols can be created on an off-line computer and transferred to the Echo system's client computer to begin a live run.

The key features of this application are described below:

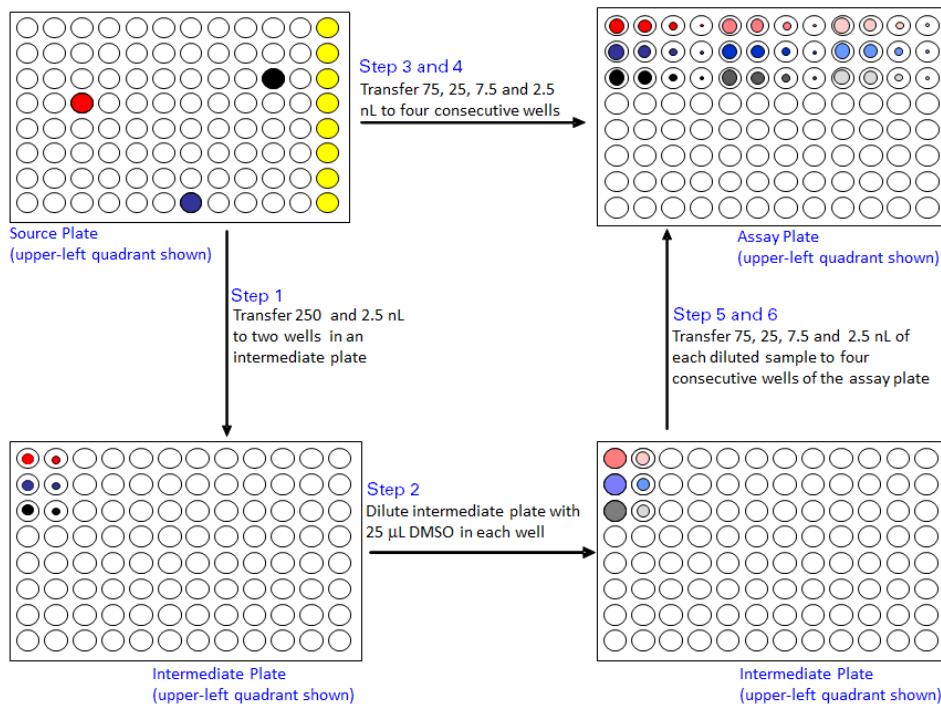
- **Sample Dilution** — Transfer of variable volumes of sample at a starting concentration to an assay plate to produce a range of sample concentration after dilution.



- Sources plates can be 384- or 1536-well formats.
- Destination plates can be a range of SLAS-compliant formats.
- Samples can consist of a range of fluid types.
- Dose-response curves can start at any location in a destination plate. Subsequent transfers can occur across rows or columns, with replicates in adjacent wells or adjacent curves.
- Dose-response curves can be interleaved.
- Multiple copies of each assay plate can be produced.
- **Intermediate dilutions** — To lengthen the concentration range or increase the number of concentration points of a curve, intermediate dilutions of the stock sample may be required. Echo Dose-Response guides the mapping of transfers from stock samples to an intermediate dilution plate. The number of intermediate dilutions required and the corresponding volume of stock sample required can be calculated with the Protocol Generator Wizard. For more information, see [Planning the Curve Data](#).
 - Source plates can be pre-filled with a diluent or solvent (DMSO for example) to dilute the stock samples to the intermediate concentration. Alternatively, intermediate plates can be diluted off-line.
- **Automated back-filling** — After a sample is transferred to the assay plate in stock and intermediate concentrations, diluent from the wells of an intermediate plate can be used to normalize the solvent concentration across the curve. Alternatively, back-filling can be performed off-line.
- **Pick lists** — Pick lists can be used to dictate the transfer of samples, controls, and intermediate concentrations to assay plates. For more information, see [Importing a Pick List](#).
- **Transfer Control** — Control reagents can be transferred from source and intermediate plates to assay plates in single or multiple concentrations.

The steps below illustrate the process used to create an intermediate plate with two additional concentrations:

1. Each stock sample is transferred from the source plate to two unique wells of the intermediate plate at 2.5 and 250 nL volumes.
2. Solvent (DMSO) is transferred to each well of the intermediate plate that contains a sample using a bulkfiller. This is an offline operation that the user or the automation system must perform. After the bulk dispensing, the user must centrifuge the plate to remove any bubbles that may be present in each of the wells.
3. The intermediate plate is replaced by the assay plate.
4. Each stock sample is transferred from the source plate to four unique wells of the assay plate at 75, 25, 7.5, and 2.5 nL volumes.
5. The source plate is replaced by an intermediate plate.
6. Each intermediate dilution is transferred from the intermediate plate to four unique wells of the assay plate at 75, 25, 7.5, and 2.5 nL volumes.



In the simplified example above, the Echo system is used to create an intermediate plate with two additional concentrations. The system then transfers samples from the source plate and intermediate plate to the assay plate. The assay plate now has sufficient sample concentrations to create a 12-pt direct dilution.

To successfully create protocols with the Echo Dose-Response application, the following steps are recommended:

- Outline Dose-Response assay requirements — Determine the required stock and intermediate volumes and concentrations required to produce the desired dose-response assay plate.
- Create Echo Dose-Response protocol — Use the information from the previous step to define layouts of the plates required for the protocol.
- Develop a back-fill strategy — Determine when to normalize solvent concentrations across a curve using the Echo Liquid Handler or an off-line dispenser.
- Simulate the protocol — Run a simulation to ensure that samples and reagents are transferred appropriately.

The steps to prepare IC50 assay plates for Echo Dose-Response application are shown below.

1. Plan the curve data. For more information, see [Planning the Curve Data](#).
2. Start a new protocol. For more information, see [Starting a New Protocol](#).
3. Create a source plate. For more information, see [Creating a Source Plate](#).
4. Use the Protocol Generator Wizard to create the intermediate plate(s) and destination plate. For more information, see [Using the Protocol Generator Wizard](#).
5. Create a fixed plate. For more information, see [Adding a Fixed Plate](#).
6. Run the protocol. For more information, see [Running a Protocol](#).
7. Import a pick list. For more information, see [Importing a Pick List](#).
8. Manage transfer data and view the reports. For more information, see [Viewing Reports](#).

Frequently Used Terms

There are many terms used throughout this guide that refer to parts of the Echo Dose-Response application. It is useful to know and understand these terms before using the application.

- **Across** — Indicates the ordering of transfers to occur in a row-wise manner.
- **Automatic back-fill** — Back-fill performed by the Echo Liquid Handler during protocol run. Settings for automatic back-filling are entered during destination plate design.
- **Back-fill** — Calculated volume of solvent added to destination wells to create a constant solvent concentration in the final assay.
- **Bulk fill** — Reservoir of solvent contained in the source, control, or intermediate plate (usually in rows or columns), to be readily available for use in back-filling during protocol run.
- **Destination plate** — Microplate receiving the transfer contents from one or more source plates.
- **Down** — Indicates the ordering of transfers to occur in a column-wise manner.
- **Fixed plate** — Echo qualified microplates containing controls or reagents for transfer to another microplate serving as a destination plate.
- **Intermediate plate** — Echo qualified source plate that contains intermediate dilutions of the stock sample or control which are used to extend the concentration range of the dose-response curve.
- **Intermediate plate template** — Mapping of the intermediate plate to show location of sample, control (optional), and solvent (optional) to a new intermediate plate.
- **Offline** — Transfers of fluids to source or assay plates not managed by the Echo Dose-Response application.
- **Online** — Transfers of fluids to intermediate or assay plates that are managed by the Echo Dose-Response application.
- **Plate Format** — Defines the physical plate based on the number of wells, plate material (for example, PP, PS), and fluid capacity (for example, LDV).
- **Plate Type** — Defines a combination of a physical plate and a fluid type. For example, 384PP_DMSO2 designates a DMSO transfer from a 384 polypropylene plate.
- **Replicate Pattern** — Replication and replicate numbering of the selected sample well to all available wells on the source plate.
- **Reserved/Available** — Option available in the Source Plate Designer window to prevent specific wells from being included in sample mapping. Reserved wells are shown as gray in the source plate template. Reserved wells can become *available* for plate mapping by selecting the reserved wells and clicking Available.
- **Sample Definition** — Description of the sample in the Source Plate Designer. Includes sample number, concentration (numeric value and units), and plate type.
- **Sample fill direction** — Maps the sample replicate in the curve to be transferred across or down a destination plate.
- **Serpentine layout** — Serpentine layout option becomes available in the Destination Plate Designer if the curve direction and sample direction match. This layout specifies how the curve wraps to the next row (when the curve direction is Across) or to the next column (when curve direction is Down) if the curve does not align evenly with the number of wells in the row or column. When using serpentine layout, the wrapping of the curve to the next column changes direction with every other row or column. Without serpentine layout, the wrapping resets to the top of the next row or column. For more details, see [Manually Creating a Destination \(assay\) Plate](#).
- **Solvent** — A liquid reagent, solution, or buffer that dissolves or dilutes another reagent.
- **Source plate** — Echo qualified microplates containing samples or reagents for transfer to another microplate serving as a *destination plate*.
- **Source plate template** — Mapping of the source plate to show location of sample, control (optional), and solvent (optional).
- **Survey** — Analysis of fluid content in the source plate performed by the Echo Liquid Handler. Analysis provides fluid volume (uL), fluid composition, and fluid height (mm). Survey can be performed on every source plate each time the protocol is run, or after survey data is expired. The user can enter survey data expiration in the Maintain Survey History dialog box in the Protocol Options window.
- **Survey history** — Record of survey data analyses. Survey history is defined in the Protocol Options window. User enters survey expiration time/day. After the survey data expires, the Echo Liquid Handler schedules a survey the next time the protocol is run.

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CHAPTER 4 | Software Operations

This chapter describes how to use the Echo Dose-Response application. It includes the following topics.

- [Planning the Curve Data](#)
- [Starting a New Protocol](#)
- [Creating a Source Plate](#)
- [Manually Creating Intermediate Plates](#)
- [Manually Creating a Destination \(assay\) Plate](#)
- [Advanced Features](#)
 - [Adding a Fixed Plate](#)
 - [Importing a Pick List](#)
- [Editing the Protocol](#)
- [Running a Protocol](#)
- [Viewing Reports](#)

Planning the Curve Data

Before starting the Echo Dose-Response application, the following information is required.

- Starting concentrations of the samples
- Layout for the source plate (location of samples, solvents, and controls as appropriate)
- Layout for the intermediate plate (location of dilutions, controls, and solvents as appropriate)
- Final assay volume
- Number of concentration points in the dose-response curve.

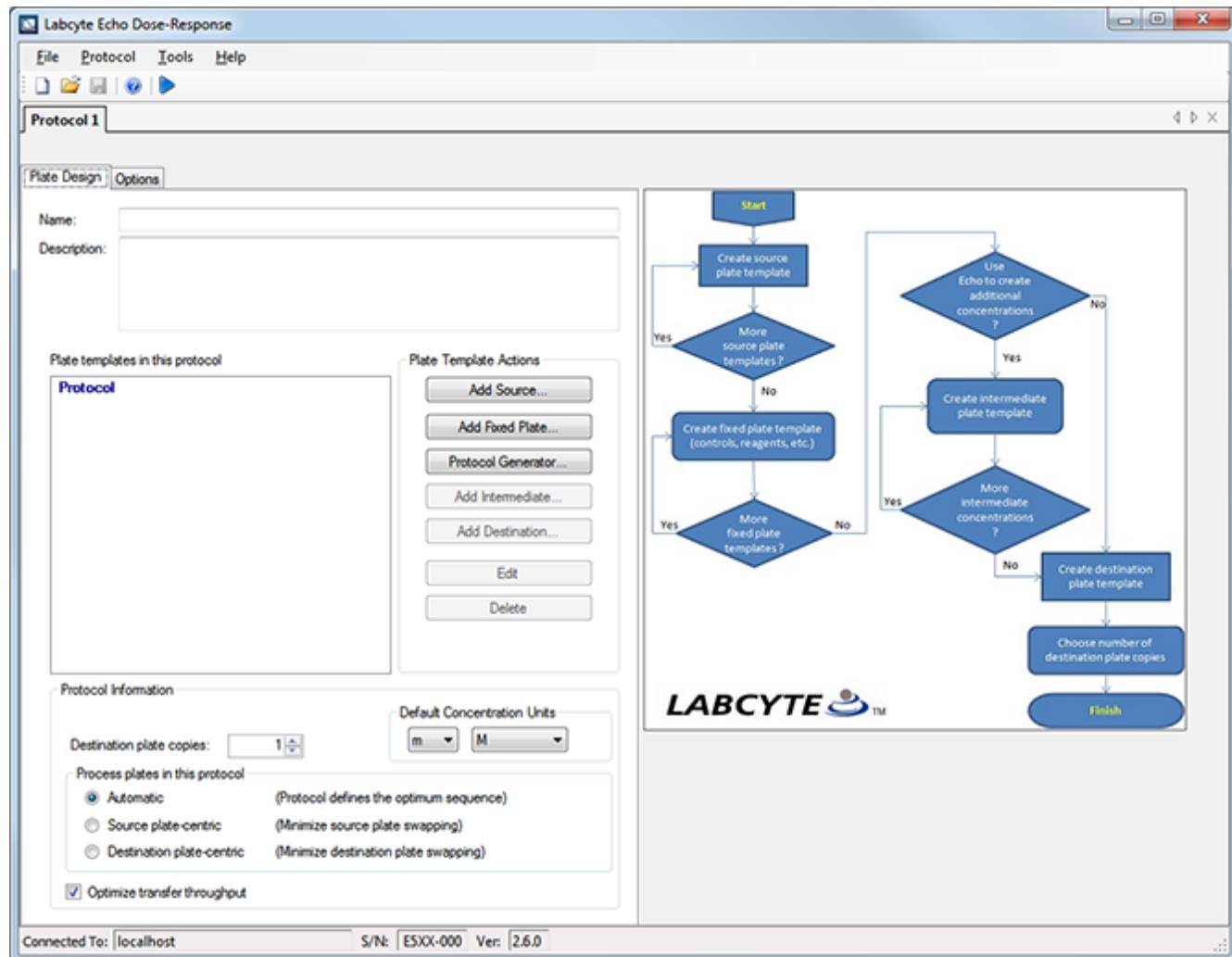
The Protocol Generator Wizard helps the user plan the curve data by determining volume requirements for samples and solvent back-fill. The wizard walks the user through the selection of parameters to define intermediate plate generation parameters and destination curve parameters. After the protocol is created using the wizard, the user can modify the protocol by re-running the wizard or by editing the individual values in each of the source, intermediate, or destination plates. However, if the user manually changes intermediate concentrations after the protocol has been generated, validation failures could result and would require manually editing the destination plate curve setup or re-running the wizard. For more information, see [Using the Protocol Generator Wizard](#).

Starting a New Protocol

There are multiple ways to start a new protocol.

1. Select **New** from the **File** menu.
2. Click the **New**  icon in the **Toolbar**.
3. Press **CTRL+N** from the keyboard.

Figure 11: Protocol Setup window



The Main Protocol window contains tabs used to design plate transfer maps and select processing options specific to the protocol.



Note: As information is entered into the template, an * appears next to the file name. This is a reminder that the file has information that has not been saved. Remember to save the protocol after designing the source plate and periodically saving thereafter.

4. Type the **Name** and **Description** of the protocol in the text fields. The description is a useful method to describe a high level outline of the protocol.

Creating a Source Plate

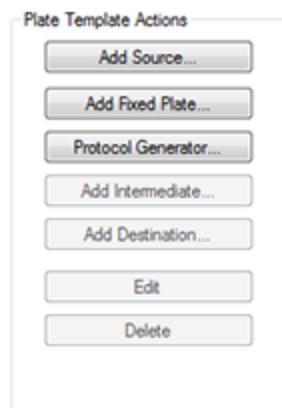
The source plate template defines the plate map of samples to be transferred to the assay plate.

To create a source plate:

1. Click **Add Source** in the **Plate Template Actions** section in the **main Protocol** window to start the **Source Plate Designer** wizard.

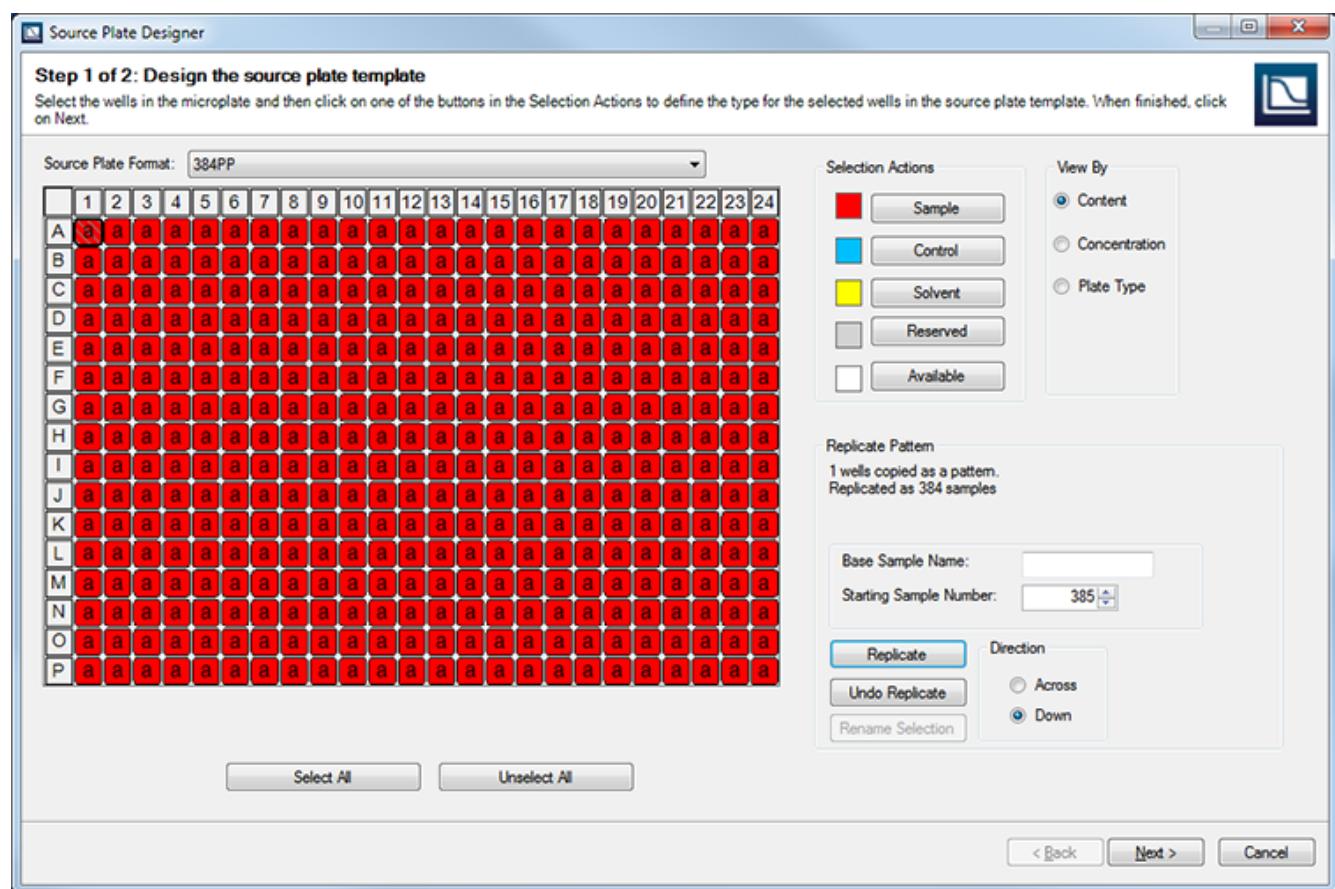
The **Source Plate Designer** wizard displays the source plate template in a new window and guides the user through each step needed to create the source plate design.

Figure 12: Plate Template Actions section



- In the **Source Plate Designer** dialog box, select the **Source Plate Format**.

Figure 13: Source Plate Designer dialog box - Step 1



Source Plate Format describes the physical properties of the plate: for example, 384 wells in a polypropylene plate (384PP).

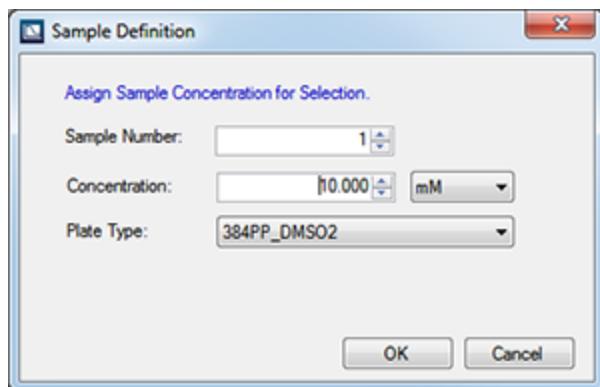


Note: The source plate must be an Echo qualified plate.

- Select the well that will contain the first sample and click the **Sample** button in the **Selection Actions** section.
- In the **Sample Definition** dialog box, enter the sample number (if different from the default), sample concentration, and units. Select the plate and fluid class from the **Plate Type** drop-down menu.

Plate Type describes the fluid type that is used by the sample. Samples with different fluid types can be assigned to the same source plate.

In this example, **384PP_DMSO2** is selected for the plate type.

Figure 14: Sample Definition dialog box

Note: The sample concentration value cannot be zero.



Note: The default concentration unit is specified in the **Main Protocol** window. The Echo Dose-Response software default concentration unit is M, but the user can change this to any of the available concentration units. Additional concentration units can be created using the **Concentration Unit Definitions** window. For more information, see [Concentration Unit Definitions](#).

5. Click **OK** to close the **Sample Definition** dialog box. The assigned well changes to red and is labeled *a*. The *a* signifies the first concentration of the sample.



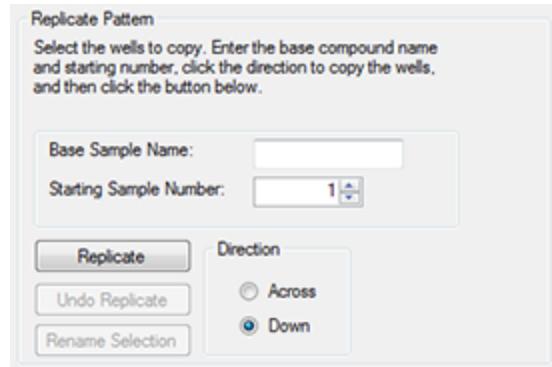
Note: To view the sample name and concentration, move the cursor over the assigned well.

6. To add another sample to the plate, repeat step 3-5. Controls can be added to the source plate and included in the dose-response curve for each sample or dispensed to specific locations on the destination plate. Solvents can be added to a source plate to use for dilution of samples and controls in a destination plate.

To replicate a sample:

1. Highlight the sample pattern to be replicated in the **Source Plate Designer** dialog box.
2. In the **Replicate Pattern** section, enter the replication direction (across or down). Optionally, enter the **Base Sample Name** and the **Starting Sample Number**. Click **Replicate**. To remove the replicate pattern created, click **Undo Replicate**.

Figure 15: Replication Pattern section

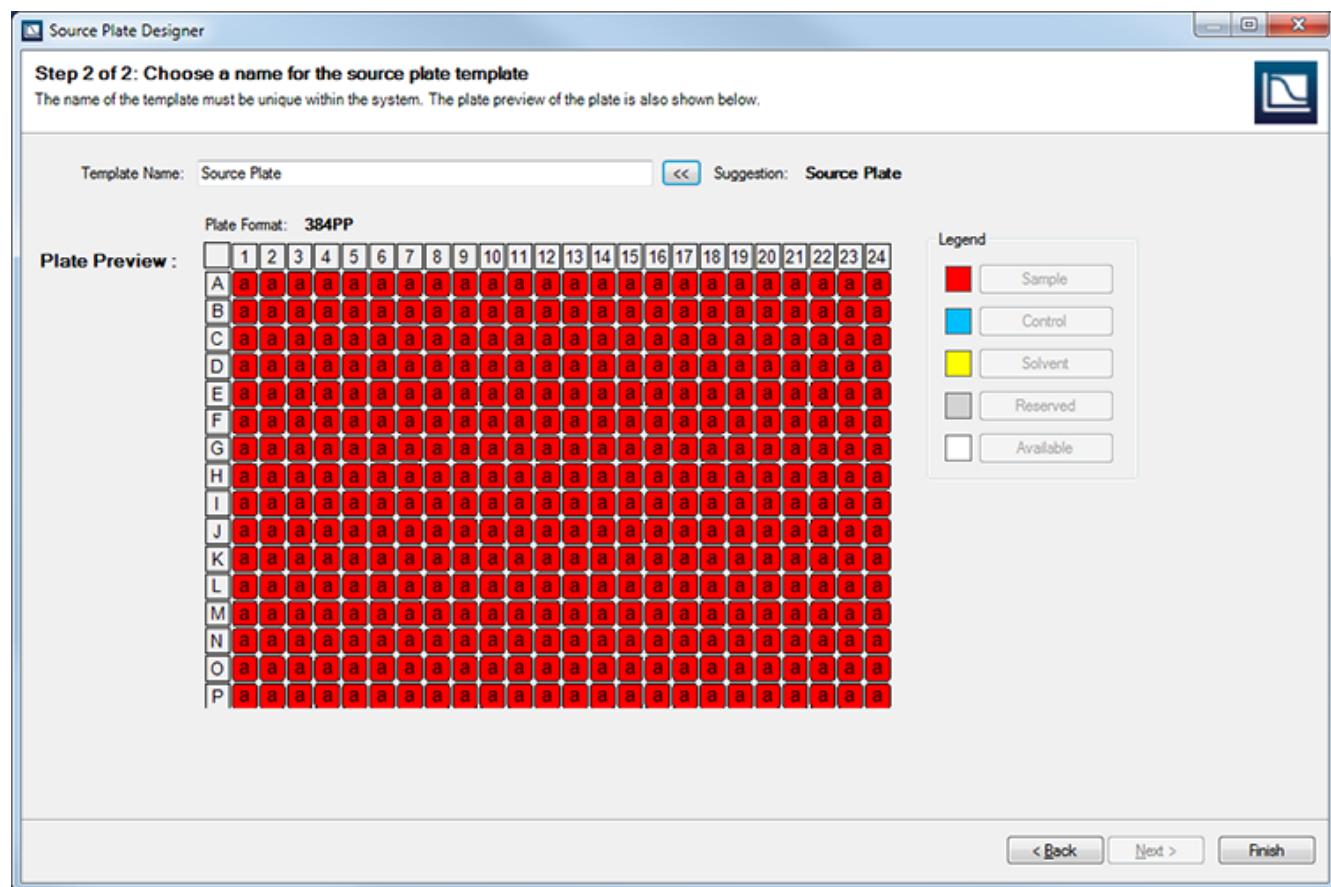


The application replicates and numbers all available wells on a plate and reports the number of replicates.



Note: To prevent replication on all available wells on the plate, click **Reserved** to reserve wells for controls or a solvent. The **Reserved** button must be clicked before the **Replicate** button to block off the desired wells.

3. Click **Next** to display the next screen and enter the source template name. Type a name in the **Template Name** field or click the left arrow button to accept the suggested name.

Figure 16: Source Plate Designer dialog box - Step 2

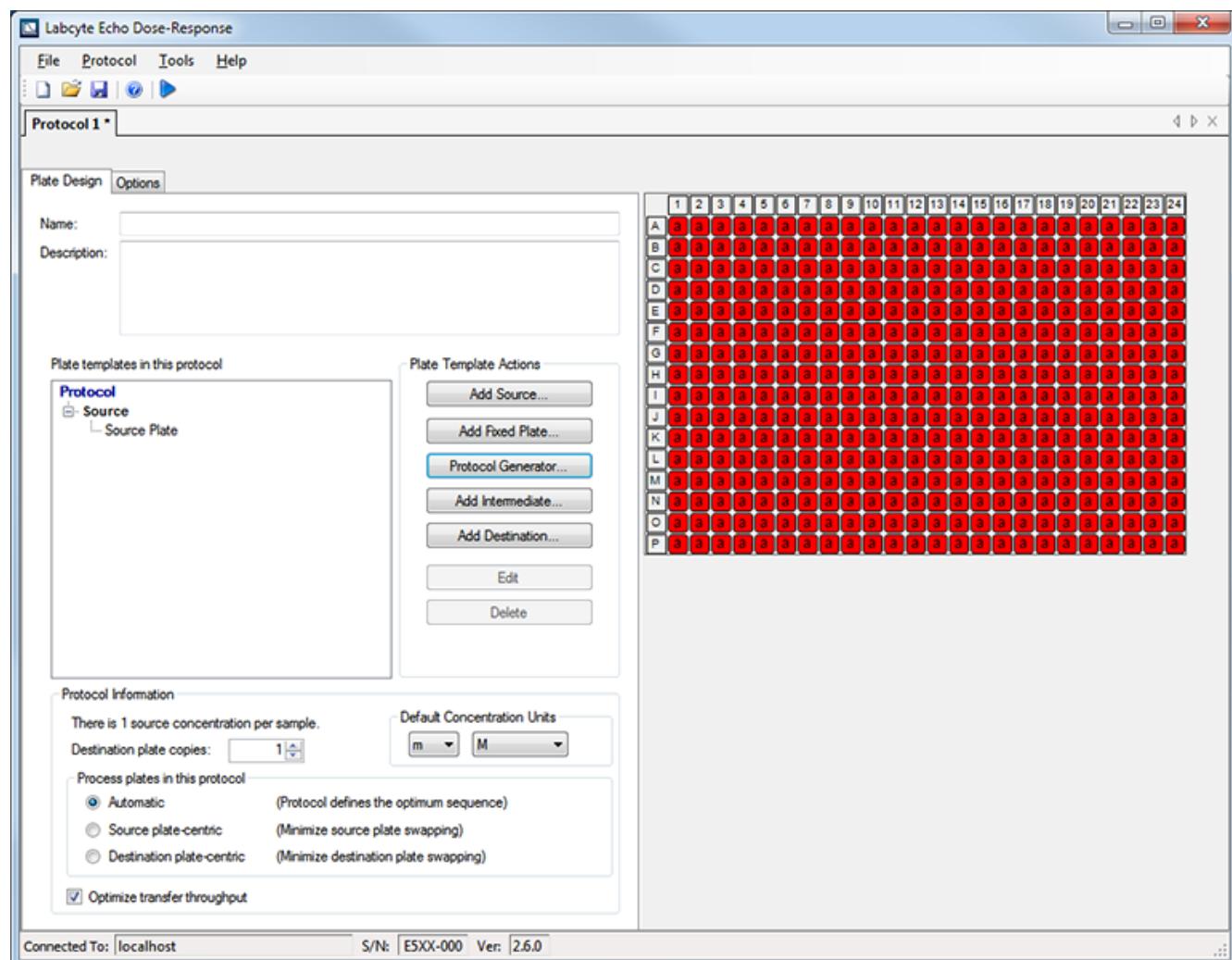
4. Click **Finish** to close the **Source Plate Designer** dialog box.

The source plate definition is complete. The **Plate Design** tab displays the design of the source plate.

5. To edit or delete the source plate template, select the source plate template in the protocol tree and click **Edit** to open the **Source Plate Designer** dialog box or click **Delete** to remove the source plate.
6. To save the protocol, select **Save** from the **File** menu and enter a file name in the prompt box. The protocol is saved with the file extension .edr in the default directory C:\Labcyte\Echo\Protocols.



Note: The protocol file can be saved to different directories. It is recommended to save the protocol often while working on it.

Figure 17: Source Plate Created

Using the Protocol Generator Wizard

The Protocol Generator Wizard helps the user plan the curve data by determining volume requirements for samples and solvent back-fill. The wizard walks the user through the selection of parameters to define intermediate plate generation parameters and destination curve parameters. After the protocol is created using the wizard, the user can modify the protocol by re-running the wizard or by editing the individual values in each of the source, intermediate, or destination plates.

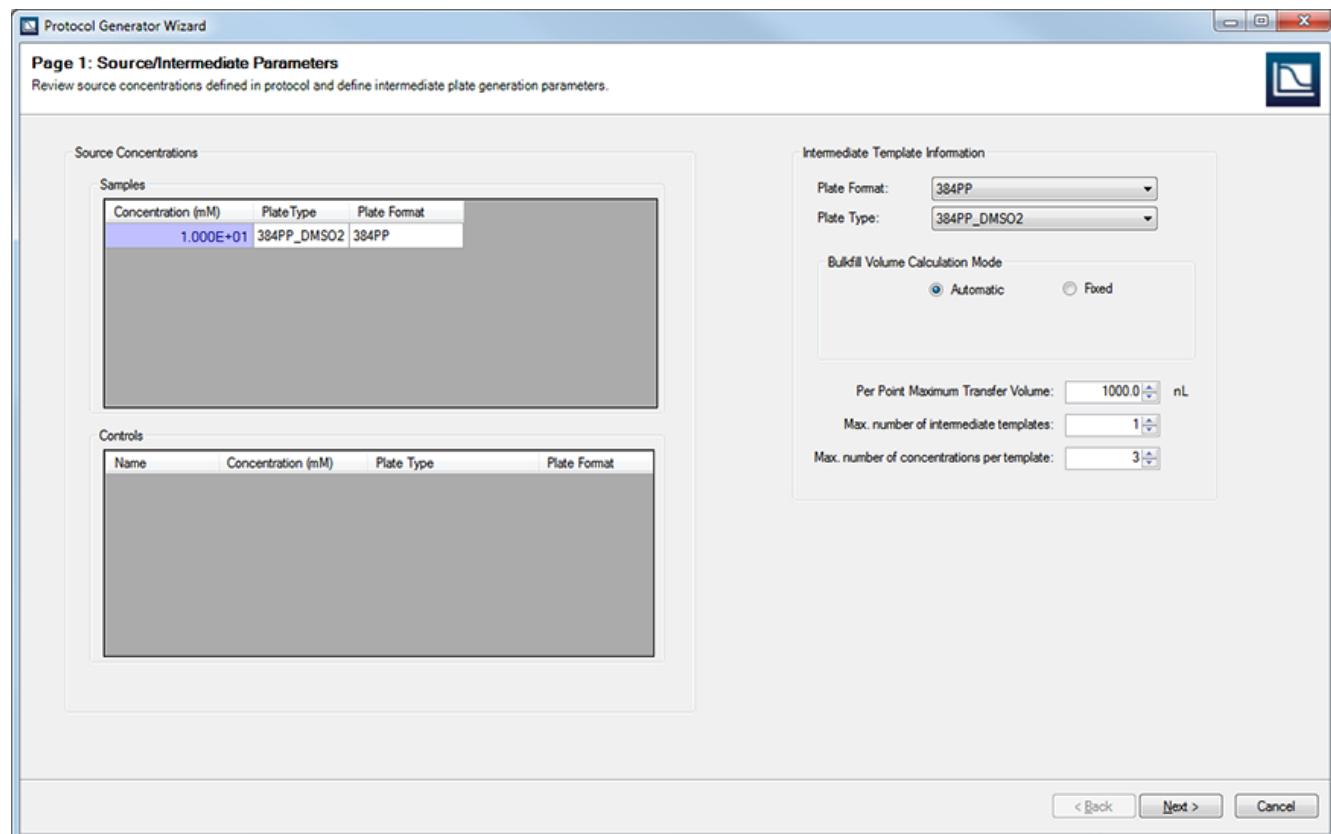
 **Note:** If the user manually changes intermediate concentrations after the protocol has been generated, validation failures could result and would require manually editing the destination plate curve setup or re-running the wizard.

Use the Protocol Generator to create Intermediate and Destination Plate(s):

1. Click **Protocol Generator** in the **Plate Template Actions** section in the **main Protocol** window to start the **Protocol Generator Wizard**. See Figure [Plate Template Actions section](#).

The **Protocol Generator Wizard** displays the source plate sample and control concentrations defined when creating the source plate in a new window and guides the user through each step needed to define the intermediate plate generation parameters.

Figure 18: Protocol Generator Step 1



2. In the **Intermediate Template Information** section, select the **Plate Format**.

Plate Format describes the physical properties of the plate: for example, 384 wells in a polypropylene plate (384PP).



Note: The intermediate plate must be an Echo qualified plate.

3. In the **Intermediate Template Information** section, select the **Plate Type**. Plate type describes the fluid type that is used when transferring from the intermediate plate to the destination plate. Selecting the plate type enables the Echo Dose-Response application to check for compatibility between the desired dilution curve and the selected plate type. Samples

with different fluid types can be assigned to the same intermediate plate. In this example, **384PP_DMSO2** is selected for the plate type.

4. In the **Bulkfill Volume Calculation Mode** section, select **Automatic** or **Fixed**.
 - **Automatic** - Should be used when the bulkfiller available allows you to specify per column bulkfill volume. The software will determine the optimal volume to be dispensed based on the volume set.
 - **Fixed** - Should be used when the bulkfiller available only allows the user to set a constant fill volume per well for the entire plate. If this option is selected, the user can set the **Constant Bulkfill Volume** for the plate.
5. In the **Intermediate Template Information** section, enter the **Per Point Maximum Transfer Volume** to indicate the highest value that is allowed to be transferred out of the source wells to make an intermediate dilution point.
6. In the **Intermediate Template Information** section, enter the **Max number of intermediate templates** to indicate the maximum number of intermediate plates from intermediate plates that will be created. Maximum allowed is 10. It is recommended that when the destination curve is very dilute relative to the starting concentration, you may need to increase the number of intermediate templates.



Note: To create a more diluted curve, you may need to increase the number of intermediate templates to further dilute the concentrations. The starting fluid concentration and ending destination fluid concentration required should be considered when setting the maximum number of intermediate templates. In addition, the order of dynamic range should be taken into account. If the destination fluid concentration required cannot be achieved with the values initially chosen, you may need to increase the number of intermediate plates.

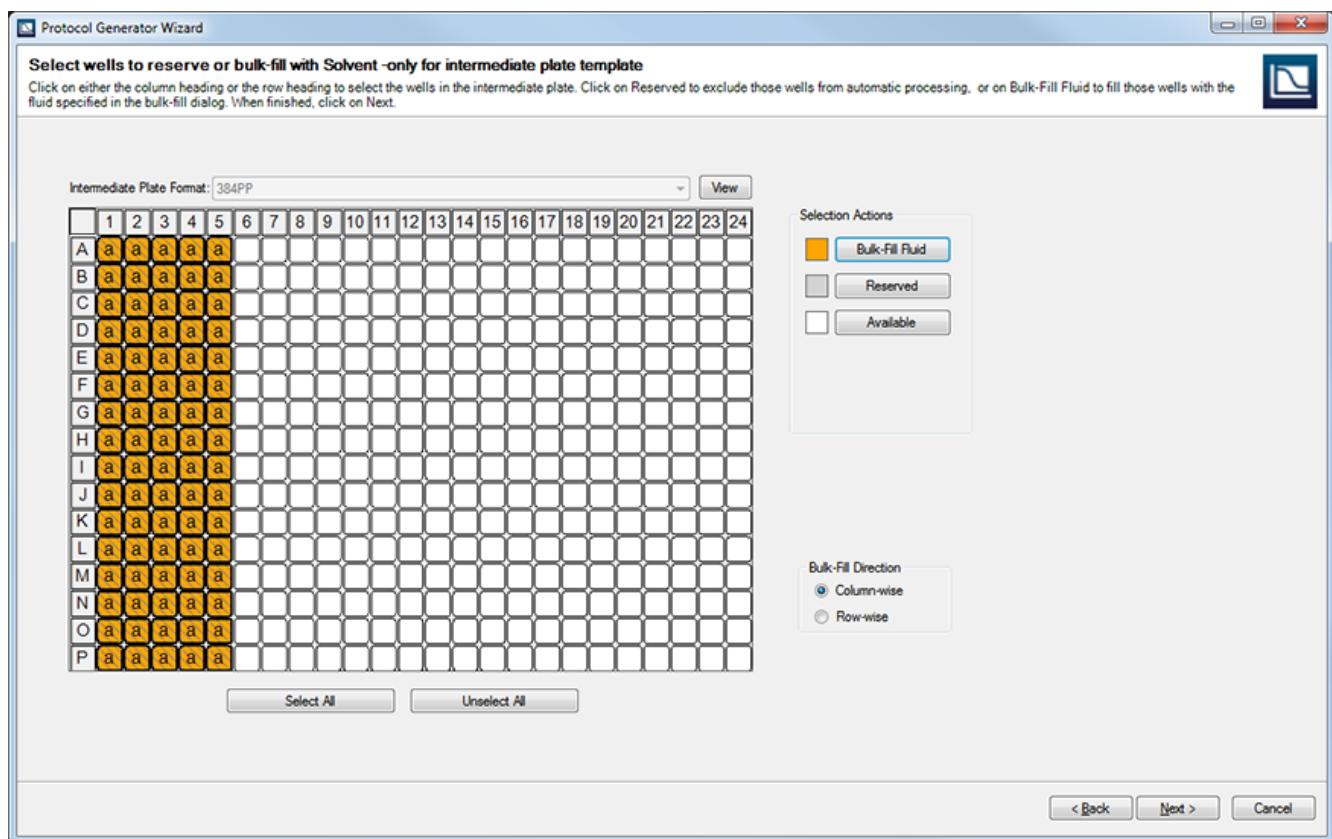
7. In the **Intermediate Template Information** section, enter the **Max number of concentrations per template** to represent the maximum number of concentration points per intermediate template used to generate the curve. Maximum allowed is 24.
8. Click **Next** to move on to page 2 of the **Protocol Generator Wizard**.

Figure 19: Protocol Generator Step 2

Page 2: Destination Curve Parameters
Define parameters used to auto-generate the destination curve for this protocol.

#	Source Concentration (mM)	Transfer Volume (nL)	Backfill Volume (nL)	Theoretical Concentration (mM)	Final Concentration (mM)	% Error Concentration	% Solvent	Comment
1	1.000E+01	250	0.0	1.000E+01	1.000E+01	0.000	100.000	
2	1.000E+01	80	170.0	3.163E+00	3.200E+00	1.170	100.000	
3	1.000E+01	25	225.0	1.000E+00	1.000E+00	-0.018	100.000	
4	3.187E-01	247.5	2.5	3.163E-01	3.155E-01	-0.260	100.000	
5	1.000E+01	2.5	247.5	1.000E-01	1.000E-01	-0.035	100.000	
6	3.187E-01	25	225.0	3.164E-02	3.187E-02	0.725	100.000	
7	3.187E-01	7.5	242.5	1.001E-02	9.560E-03	4.654	100.000	
8	3.187E-01	2.5	247.5	3.164E-03	3.187E-03	0.708	100.000	
9	1.020E-03	245	5.0	1.001E-03	9.999E-04	-0.080	100.000	
10	1.020E-03	77.5	172.5	3.165E-04	3.163E-04	-0.058	100.000	

9. In the **Destination Curve Parameters** section, set the **Curve Type** to **Custom** or **Fold-based**.
 - Use **Custom** to manually define the curve in the **Theoretical** tab.
 - Use **Fold-based** to calculate the curve based on the following values:
 - **Starting Concentration** - The first theoretical concentration.
 - **Dilution Factor** - The factor used to calculate the next concentration. The formula used is $Tc(i+1) = Tc(i)$
 - **Data Points** - The number of data points in the dose-response curve. This value is used to generate the number of rows in the **Theoretical** tab, **Actual** tab, and the **Curve Display** tab.
10. In the **Destination Curve Parameters** section, set the **Added Assay Reagent Value** to the volume to be added to each destination well.
11. In the **Destination Curve Parameters** section, set the **Per Point Maximum Transfer Volume** to the maximum transfer volume from the source or intermediate plate well to the destination well.
12. In the **Destination Curve Parameters** section, set the **Maximum Solvent Percentage** to indicate the maximum solvent percentage allowed.
13. In the **Destination Curve Parameters** section, set the **Per Point Concentration Tolerance** to indicate the maximum concentration error from the theoretical concentration allowed.
14. In the **Destination Curve Parameters** section, set the **Number of Curve Replicates** to indicate the number of times a curve should be replicated or transferred for a given sample.
15. In the **Destination Curve Parameters** section, set the **Number of Destination Copies** to indicate the number of destination plate copies that should be generated. This setting allows the user to limit transfer volume from a source to destination well.
16. Click **Generate Table** to update the tabs for **Theoretical**, **Actual**, and **Curve Display** tabs. In addition, a tab is created for each intermediate plate. The tabs are described below:
 - **Theoretical** - Displays a table with the concentration points for the curve.
 - **Actual** - Displays a table with the theoretical and actual concentration points for the curve.
 - **Curve Display** - Displays a graph of the concentration curve in a linear and logarithmic form.
 - **Intermediate** - Displays a tab for each intermediate plate that shows a table with the concentration points for that intermediate plate.
17. In the **Automatic Backfill** section, set **Perform Backfill** to **First**, **Last** or **None**.
 - **First** - Perform backfill before samples are transferred to the destination plate.
 - **Last** - Perform backfill after all the samples are transferred to the destination plate.
 - **None** - No backfill will be performed.
18. In the **Automatic Backfill** section, set the **Maximum Backfill Volume** to indicate the maximum amount of backfill volume allowed.
19. In the **Automatic Backfill** section, select the checkbox for **Backfill offline for volumes greater than/equal to** and set a value to indicate the volume at which offline backfill should be performed. If the backfill volume is greater than the volume indicated and the checkbox is selected, an offline backfill is performed. Otherwise, only online backfill is performed.
20. Click **Next** to move on to the next page of the **Protocol Generator Wizard**. This part of the wizard walks the user through the steps to create an intermediate plate.

Figure 20: Protocol Generator Step 3

21. Select a **Bulk-Fill Direction** option (column-wise or row-wise) before selecting rows or columns containing solvent.



Note: Changing the bulk-fill direction requires the user to re-select the wells containing the bulk-fill solvent.



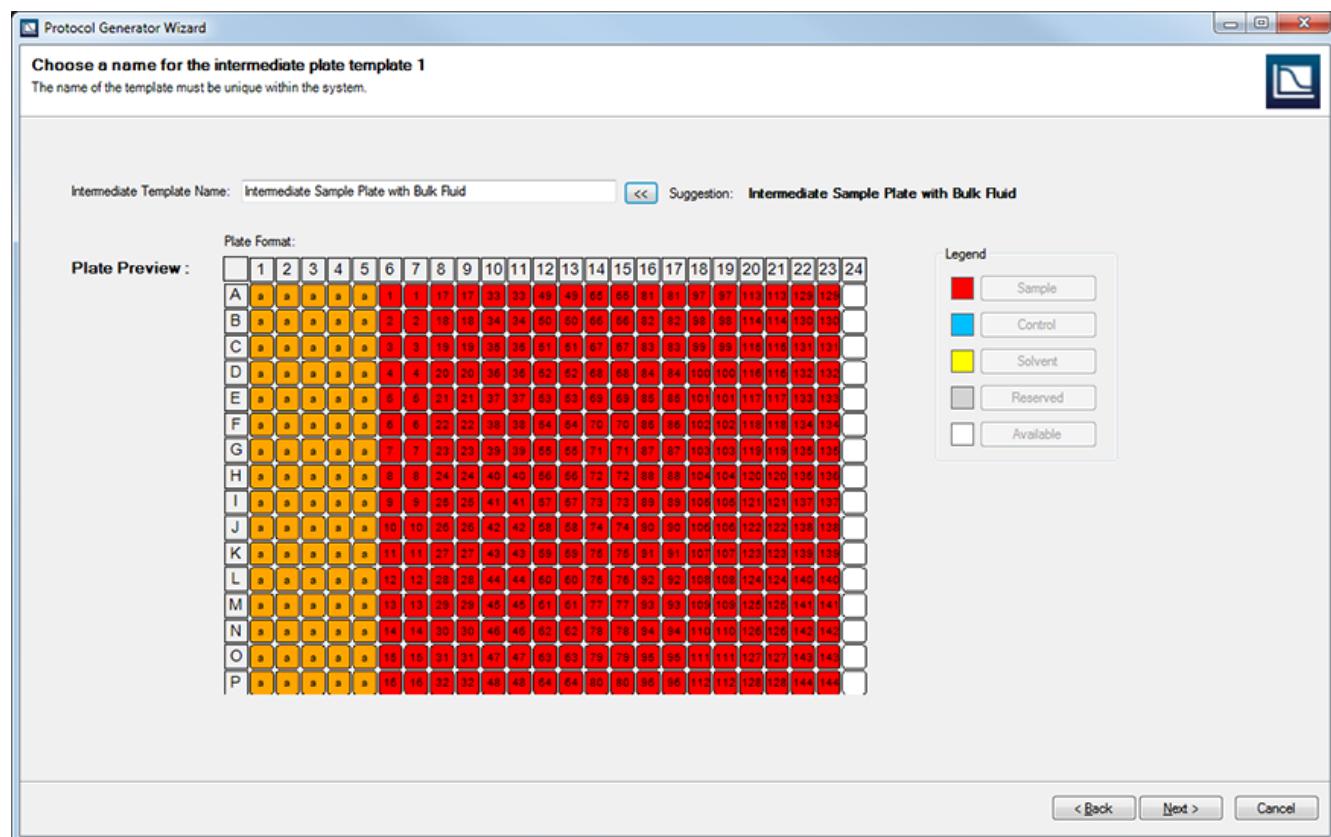
Note: During plate preparation or during the protocol run when the bulk-fill screen is displayed, the user will fill the intermediate plate with solvent, either manually or with a bulk filler.

22. Based on the bulk-fill direction chosen, select the rows or columns of wells that will contain solvent and click **Bulk-Fill Fluid**.
23. Based on the bulk-fill direction chosen, select the rows or columns of wells that will be excluded from automatic processing and click **Reserved**.

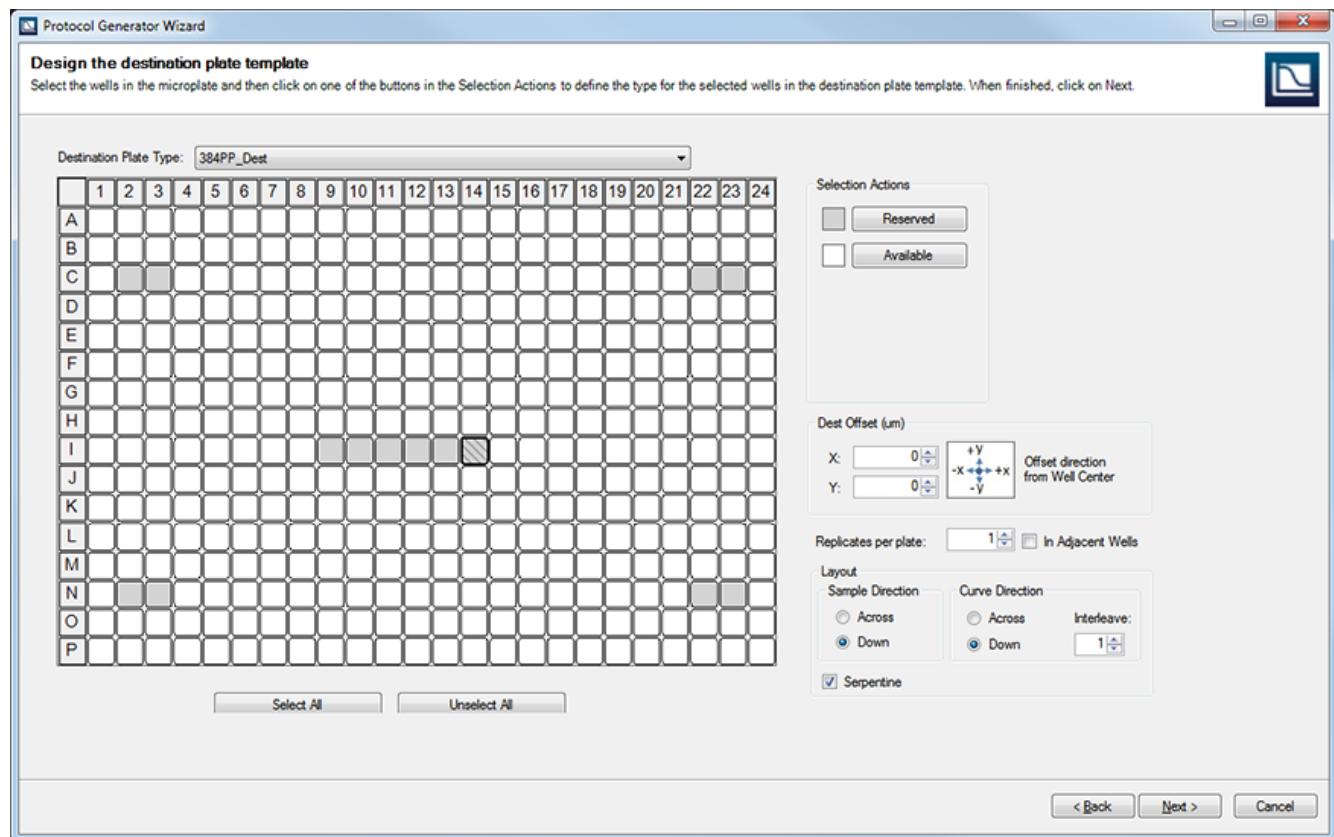


Note: To unselect wells that were previously selected as **Reserved** or for **Bulk-Fill Fluid**, select the rows or columns of wells and click **Available**.

24. Click **Next** to continue to the final step in creating the intermediate plate by entering a unique **Intermediate Template Name**.

Figure 21: Protocol Generator Step 4

25. Click **Next** to move on to the next page of the **Protocol Generator Wizard**. This part of the wizard walks the user through the steps to create the destination plate.

Figure 22: Protocol Generator Step 5

26. Select the desired **Destination Plate Type** from the list.
27. Select the wells that will be reserved and click **Reserved**.
28. Select the wells that will be available for bulk fill and click **Available**.
29. Optionally, if you want to move where the drop should land during a transfer, set the values for **Dest Offset** for horizontal (X) value and the vertical (Y) value for the offset of the drop. A positive number indicates the offset to move to the right or up and a negative number indicates the offset to move to the left or down. The default drop location when a transfer is performed is the center of the destination well.
30. Enter a value for **Replicates per plate**. More than one replicate can be mapped in numeric order or grouped by the sample number by checking **In Adjacent Wells**.
31. Select the **Sample Direction** (Across or Down).
32. Select the **Curve Direction** (Across or Down) and set the value for **Interleave**.

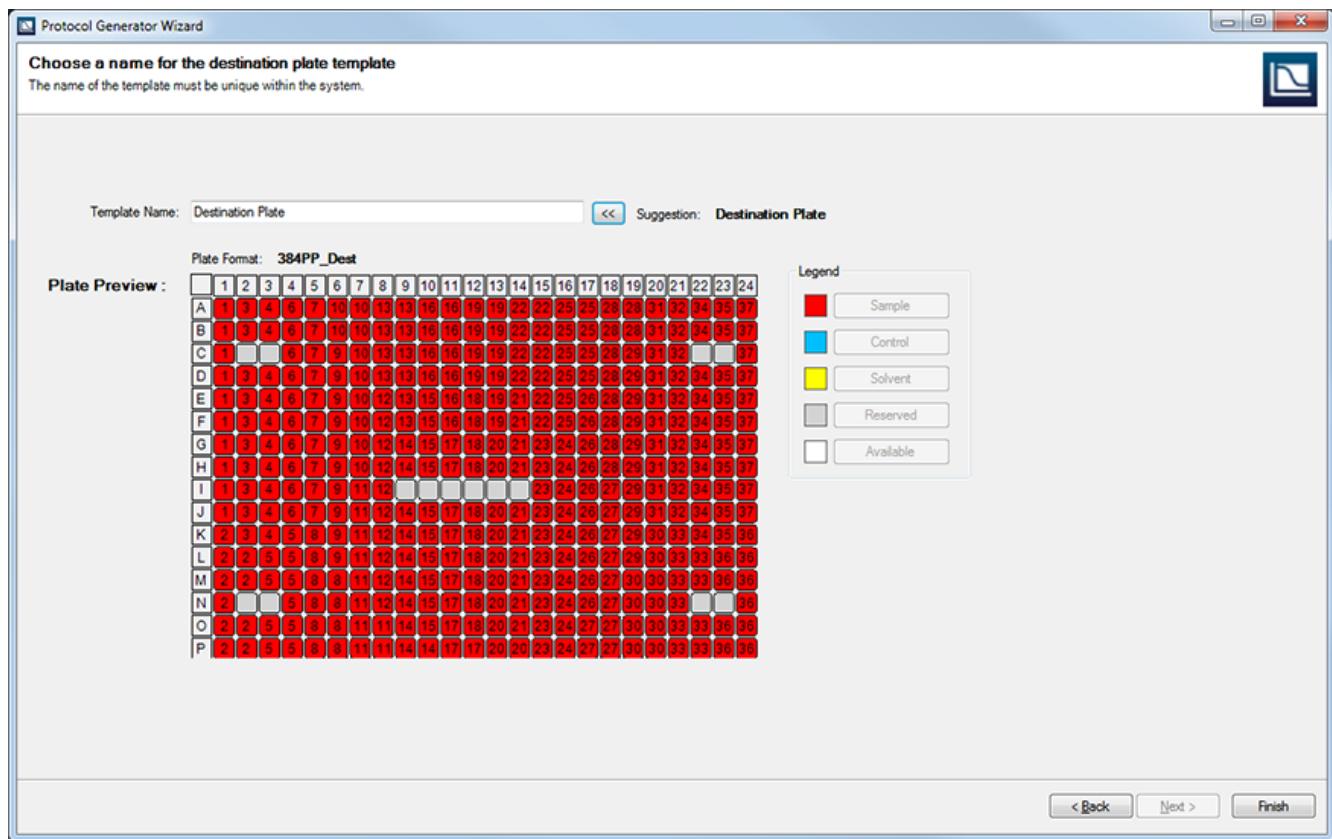


Note: The Dose-Response application fits all the sample concentrations for a curve on the same plate if possible. If a complete curve cannot fit on the plate, it is moved to the next plate.

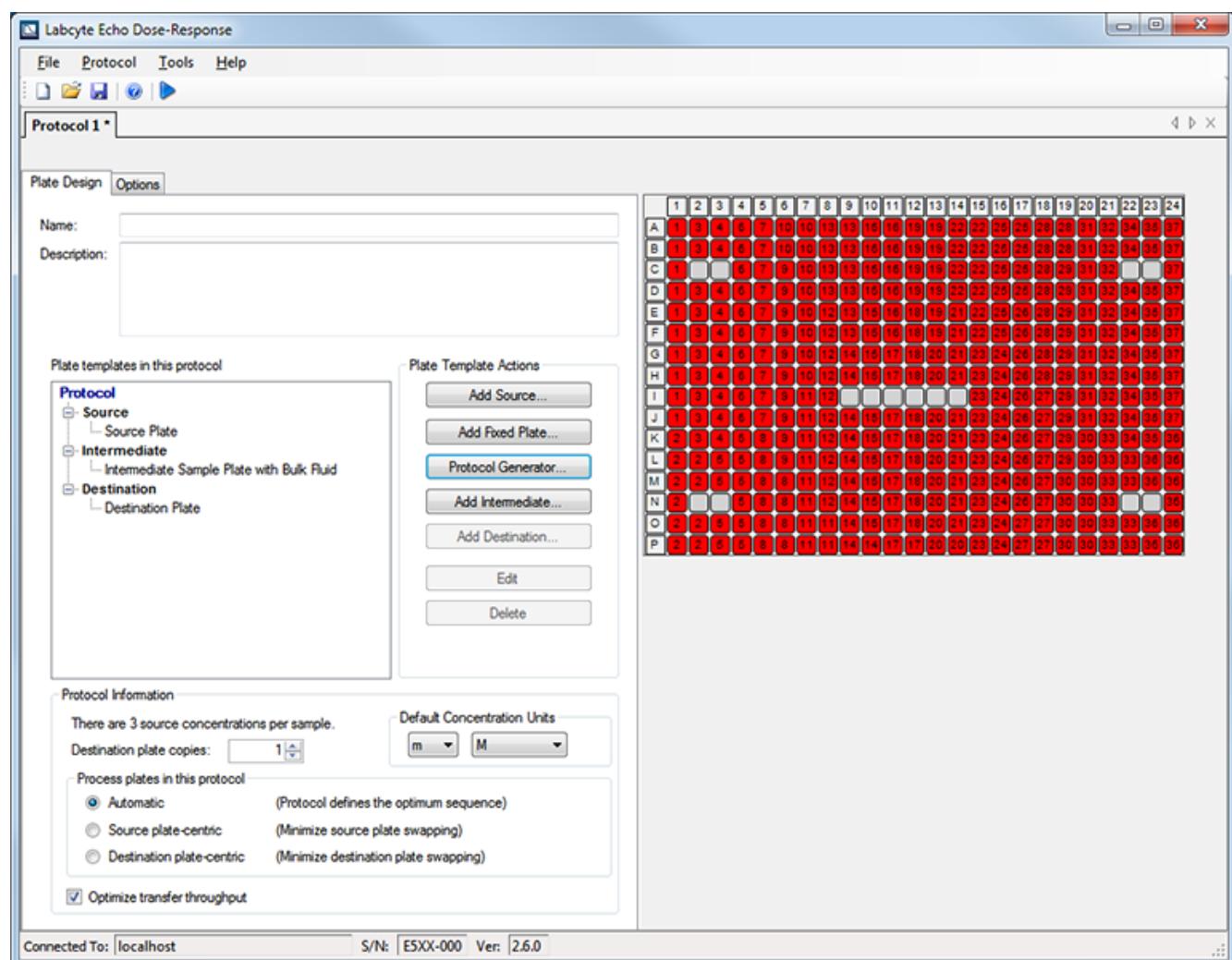
33. If the **Sample Direction** and **Curve Direction** match, the **Serpentine** checkbox becomes available. This layout specifies how the curve wraps to the next row (when the curve direction is Across) or to the next column (when curve direction is Down) if the curve does not align evenly with the number of wells in the row or column. When using Serpentine layout, the wrapping of the curve to the next column changes direction with every other row or column. Without Serpentine layout, the wrapping resets to the top of the next row or column. Select the **Serpentine** checkbox if desired.

34. Click **Next** to continue to the final step to enter a unique **Destination Template Name**.

Figure 23: Protocol Generator Step 6



35. Click **Finish** to close the **Protocol Generator Wizard** dialog box. The destination plate definition is complete. The **Plate Design** tab displays the design of the destination plate.

Figure 24: Main Window after running the Protocol Generator Wizard

Manually Creating Intermediate Plates

If the source plates only contain samples, the **Intermediate Plate Designer** wizard will be three steps. If the source plates contain samples and controls, the **Intermediate Plate Designer** wizard will be four steps; an additional step is added to build the intermediate plate for controls.

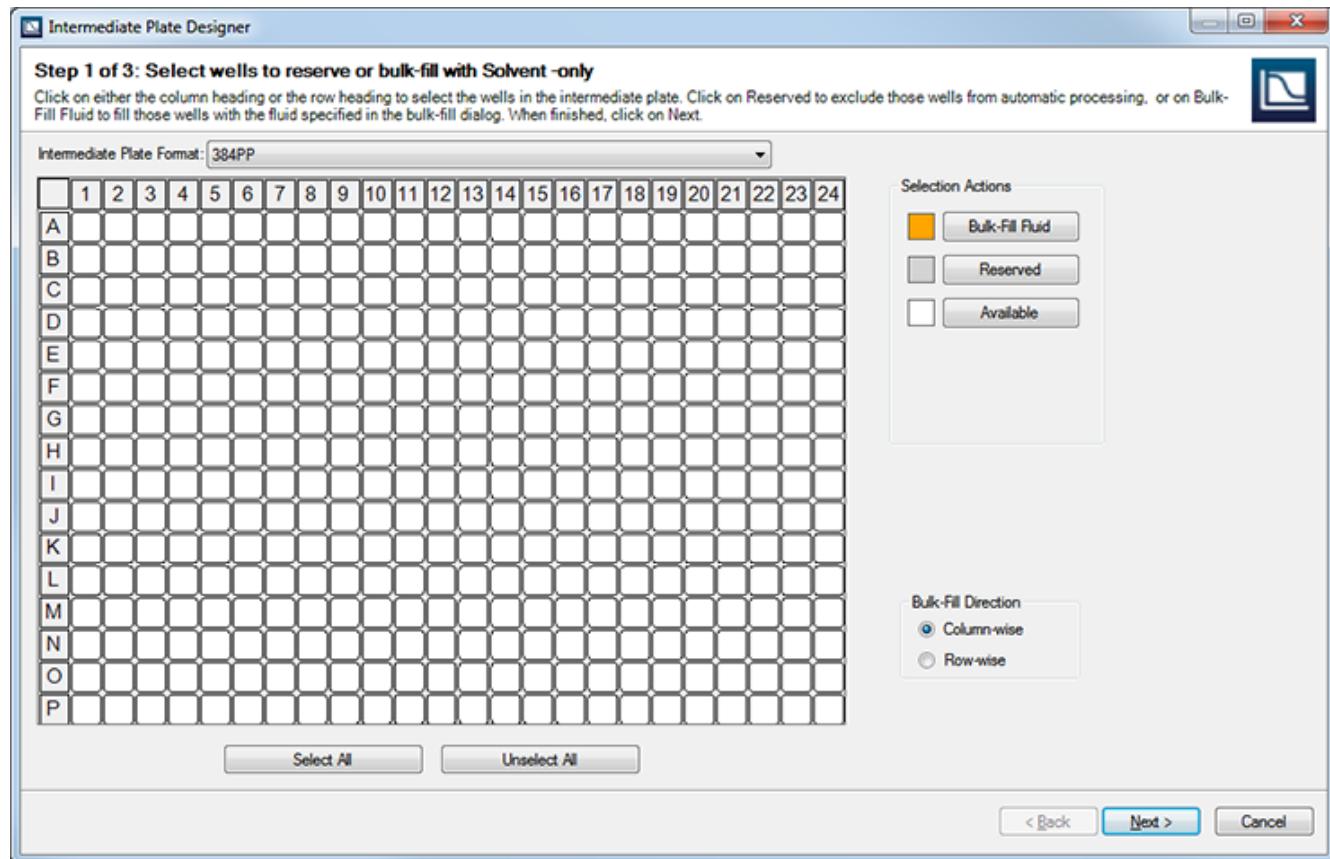
 **Note:** It is recommended to use the **Protocol Generator Wizard** to create intermediate plates rather than creating intermediate plates manually. For more information, see [Using the Protocol Generator Wizard](#).

To create an intermediate plate:

1. Click **Add Intermediate** in the **Plate Template Actions** section in the **main Protocol** window to start the **Intermediate Plate Designer** wizard. See Figure [Plate Template Actions section](#).

The **Intermediate Plate Designer** wizard displays the intermediate plate template in a new window and guides the user through each step needed to create the intermediate plate design.

Figure 25: Intermediate Plate Designer dialog box - Step 1



2. In the **Intermediate Plate Designer** dialog box, select the **Intermediate Plate Format**.

Intermediate Plate Format describes the physical properties of the plate: for example, 384 wells in a polypropylene plate (384PP).



Note: The intermediate plate must be an Echo qualified plate.



Note: If the intermediate plate will contain solvent to back-fill and normalize points of the dose-response curve, use steps 3 and 4 to indicate the location of the solvent wells.

3. Select a **Bulk-Fill Direction** option (column-wise or row-wise) before selecting rows or columns containing solvent.



Note: Changing the bulk-fill direction requires the user to re-select the wells containing the bulk-fill solvent.



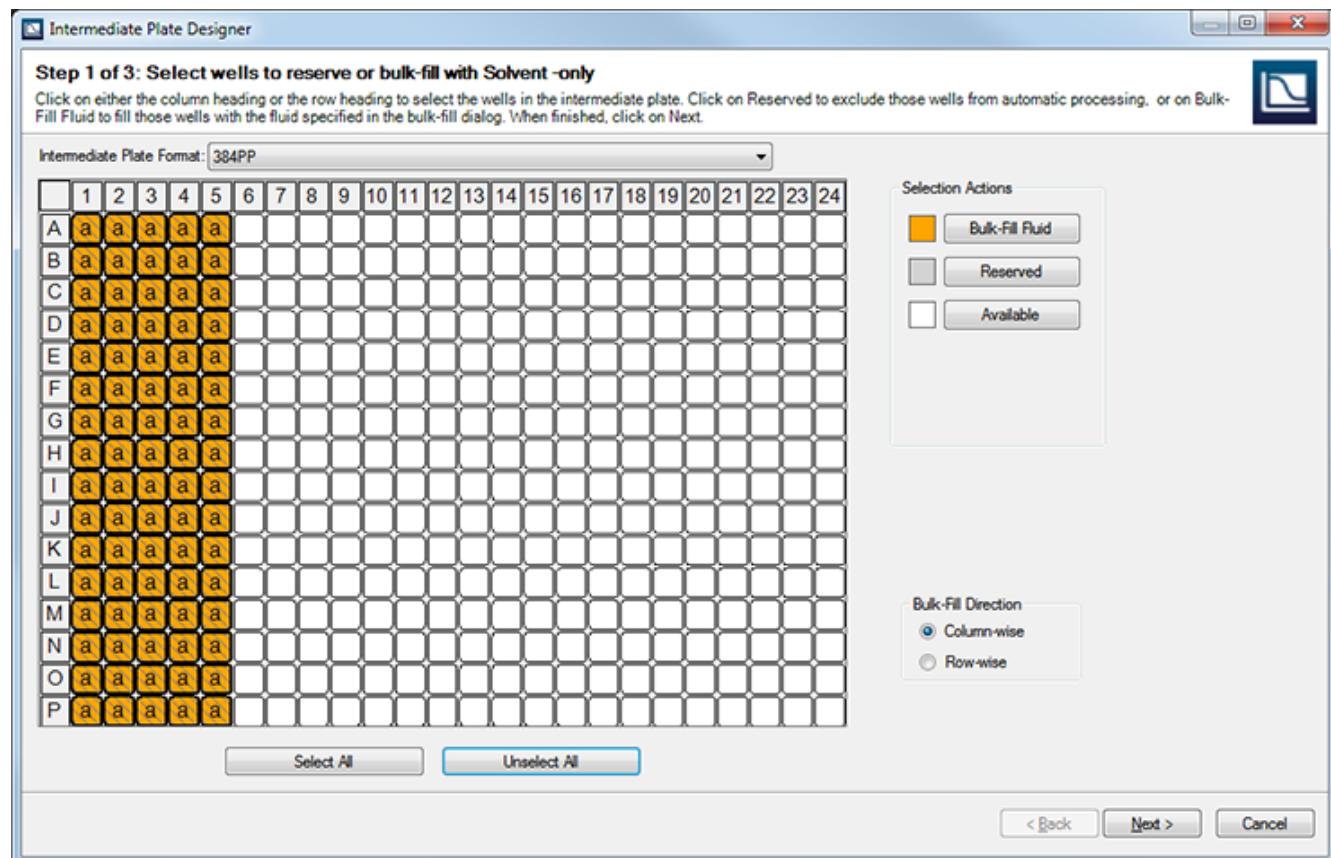
Note: During plate preparation or during the protocol run when the bulk-fill screen is displayed, the user will fill the intermediate plate with solvent, either manually or with a bulk filler.

4. Based on the bulk-fill direction chosen, select the rows or columns of wells that will contain solvent and click **Bulk-Fill Fluid**.
5. Based on the bulk-fill direction chosen, select the rows or columns of wells that will be excluded from automatic processing and click **Reserved**.



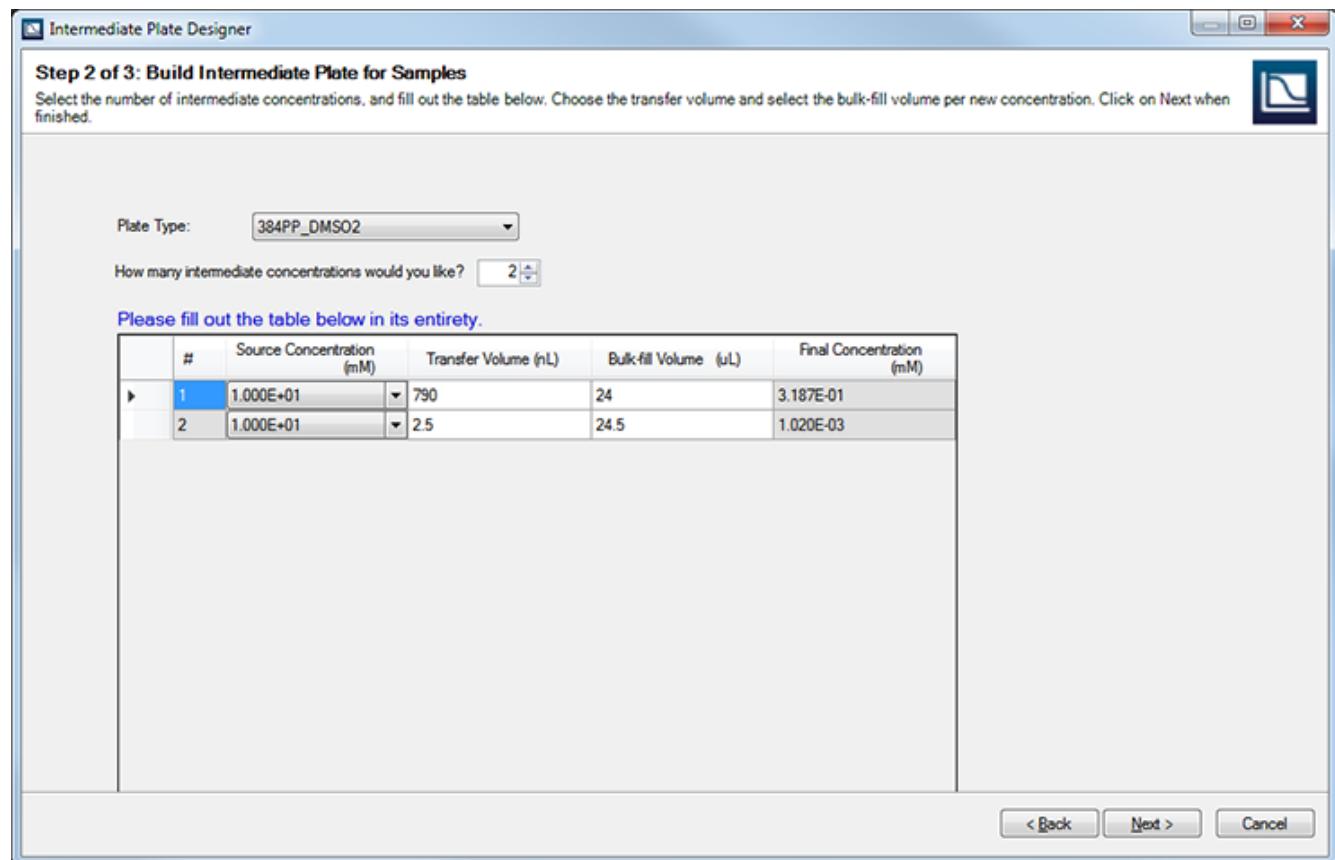
Note: To unselect wells that were previously selected as **Reserved** or for **Bulk-Fill Fluid**, select the rows or columns of wells and click **Available**.

Figure 26: Intermediate Plate Designer dialog box - Step 1



- Click **Next** to continue to the next step to calculate the intermediate sample concentrations.

Figure 27: Intermediate Plate Designer dialog box - Step 2



- In the **Intermediate Plate Designer** dialog box, select the **Plate Type**.

- Enter the number of intermediate concentrations to be created.



Note: The value entered for intermediate concentrations determines the number of lines (or rows) in the table.

- In the table for intermediate concentrations, select the sample concentration from the **Source Concentration** list and enter corresponding values for **Transfer Volume** and **Backfill Volume**. The value for **New Concentration** is calculated by the Dose-Response application. This step should be completed for each row in the table.



Note: The range of the new concentrations created depends on the working range of the source plate selected. For example, the 384PP_DMSO2 plate has a working range of 15 μL to 65 μL.

For more information on the working ranges for other source plates, go to <http://www.labcyte.com/products/echo-qualified-microplates>.

- Click **Next** to continue to the next step.



Note: If the source plate contains controls with concentrations or if the **Protocol Generator Wizard** was not used, follow the three steps below to create the intermediate control concentrations. Otherwise, the **Intermediate Plate Designer** continues to the final step.

1. In the **Intermediate Plate Designer** dialog box, select the **Plate Type**.
2. Enter the number of intermediate control concentrations and intermediate control samples to be created.



Note: The value entered for intermediate concentrations controls the number of wells added in the table.

3. In the table for intermediate concentrations, select the sample concentration from the **Source Concentration** list and enter corresponding values for **Transfer Volume** and **Bulk-fill Volume**. The value for **New Concentration** is calculated by the Dose-Response application. This step should be completed for each row in the table.

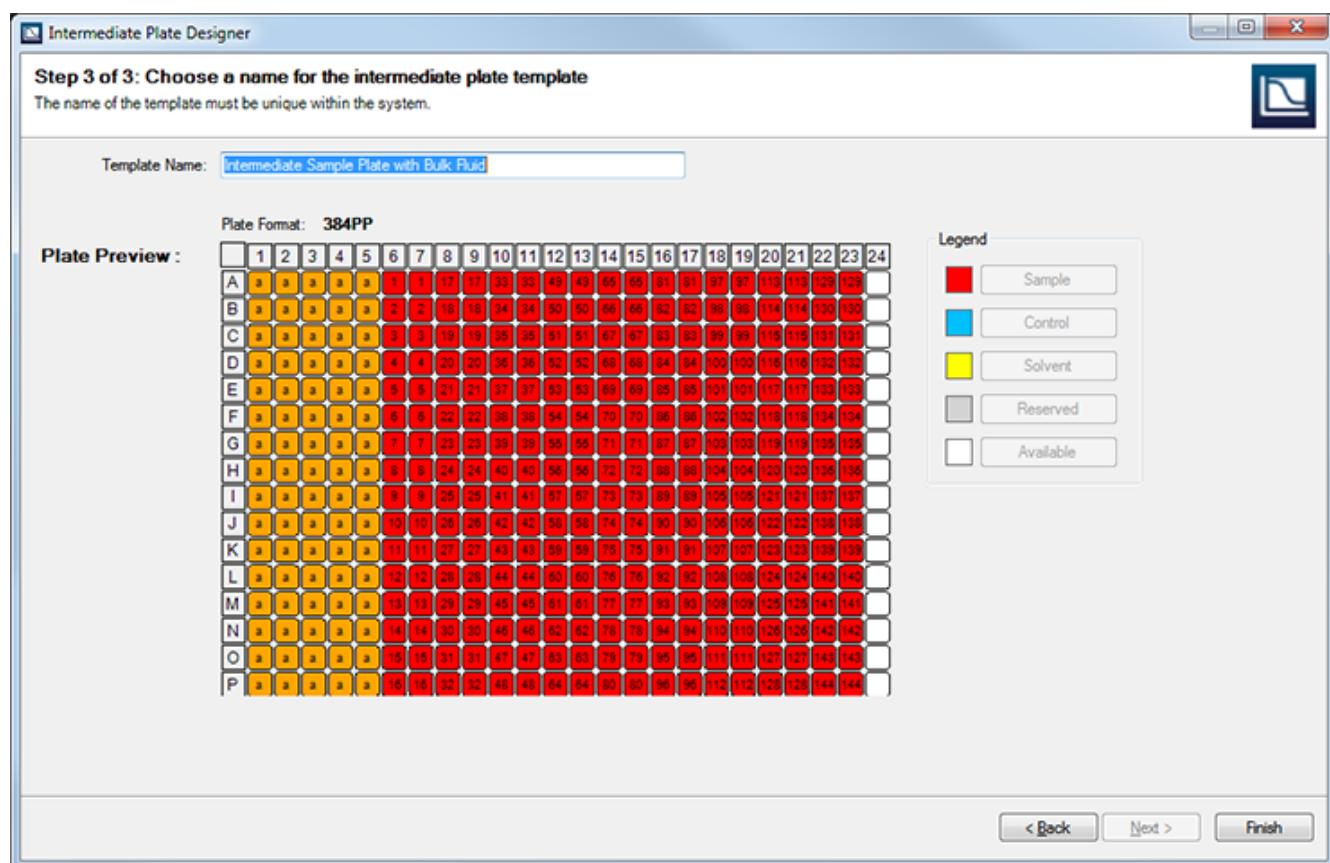


Note: The range of the new concentrations created depends on the working range of the source plate selected. For example, the 384PP_DMSO2 plate has a working range of 15 µL to 65 µL.

For more information on the working ranges for other source plates, go to <http://www.labcyte.com/products/echo-qualified-microplates>.

11. Click **Next** to continue to the final step to enter a unique intermediate **Template Name**.

Figure 28: Intermediate Plate Designer dialog box - Step 3



12. Click **Finish** to close the **Intermediate Plate Designer** dialog box.

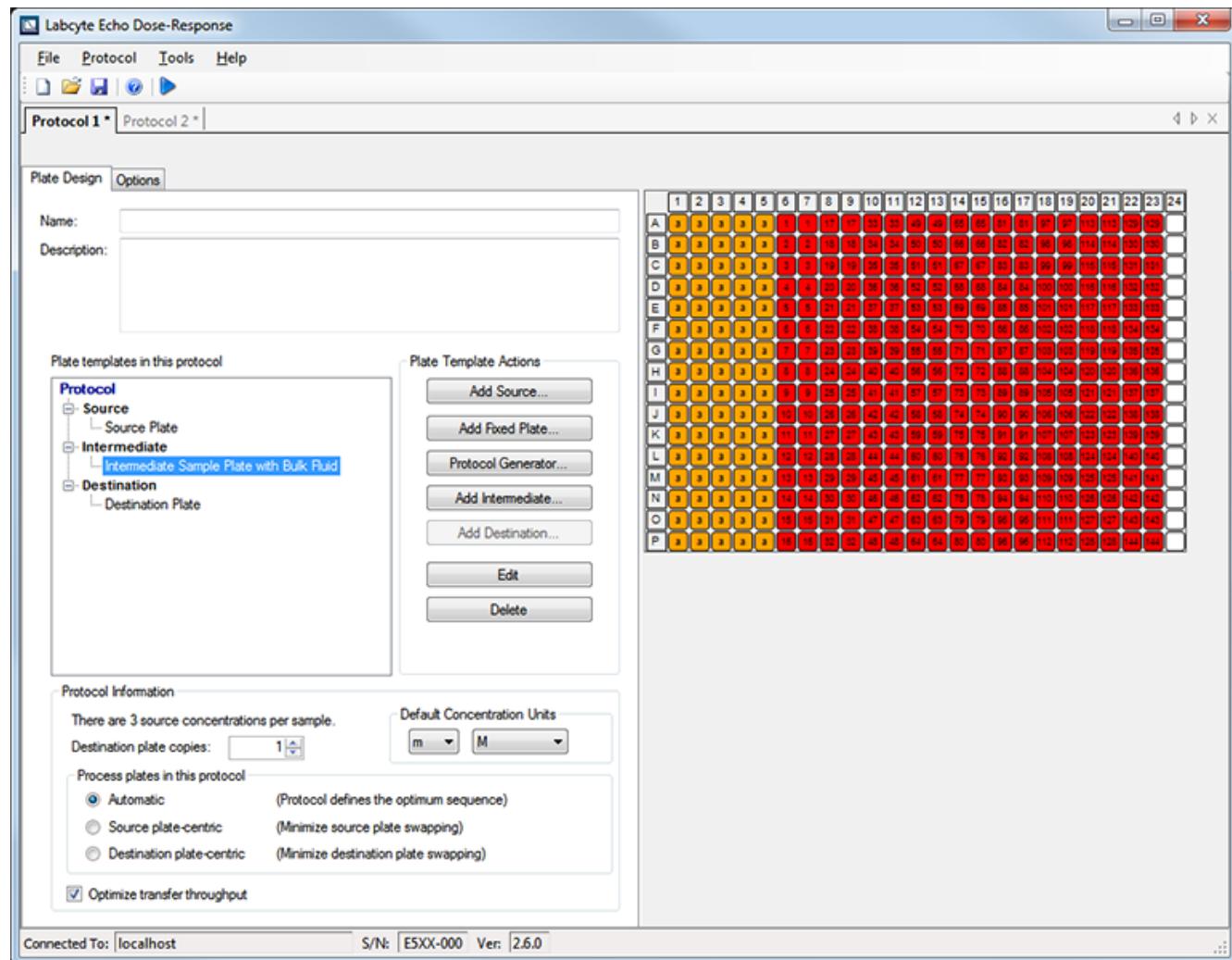
The intermediate plate definition is complete. The **Plate Design** tab displays the design of the intermediate plate.

13. To edit or delete the intermediate plate template, select the intermediate plate template in the protocol tree and click **Edit** to open the **Intermediate Plate Designer** dialog box or click **Delete** to remove the intermediate plate.
14. To save the protocol, select **Save** from the **File** menu and enter a file name in the prompt box. The protocol is saved with the file extension .edr in the default directory C:\Labcyte\Echo\Protocols.



Note: The protocol file can be saved to different directories. It is recommended to save the protocol often while working on it.

Figure 29: Intermediate Plate Created



Creating Intermediate Plates From Existing Intermediate Plates

Concentrations created in an intermediate plate that are used to produce new concentrations in subsequent intermediate plates are sometimes referred to as daisy chaining plates.

This is required for concentration curves where the concentration range is so large that all the intermediate concentrations required cannot be made from the stock sample.

The steps below describe how to create a second intermediate plate that uses the concentrations built in the first intermediate plate.

To create an intermediate plate from an existing intermediate plate:

1. Create initial intermediate plate. Follow steps in [Manually Creating Intermediate Plates](#).
2. Repeat steps 1- 8, in [Manually Creating Intermediate Plates](#) to create a secondary intermediate plate.
3. In step 9, in [Manually Creating Intermediate Plates](#), when the **Source Concentration** list is selected, the concentrations created in the initial intermediate plate are listed. These intermediate concentration values can be used to further dilute the subsequent concentrations.

#	Source Concentration (nM)	Transfer Volume (nL)	Bulk-fill Volume (μL)	Final Concentration (nM)
1	1.000E+01	2.5	11	2.272E-03
2	2.272E-03	2.5	11	5.163E-07
	1.000E+01 2.272E-03 5.986E-05			

4. Complete the remaining steps to complete the creation of a secondary intermediate plate.

Reusing Intermediate Plates Across Multiple Protocols

Once an intermediate plate is created, the intermediate concentrations on that plate can be reused in multiple protocols via importing a pick list. For more information, see [Importing a Pick List](#).

Manually Creating a Destination (assay) Plate

When creating a destination plate, a solvent can be transferred to specific wells, controls can be defined, and wells can be reserved. Each of these actions is explained in the steps below.

Note: It is recommended to create a destination plate using the **Protocol Generator Wizard**. See the [Using the Protocol Generator Wizard](#) topic for more information. However, once a source plate has been created, a user can create a destination plate using the **Add Destination** button. This process is explained below.

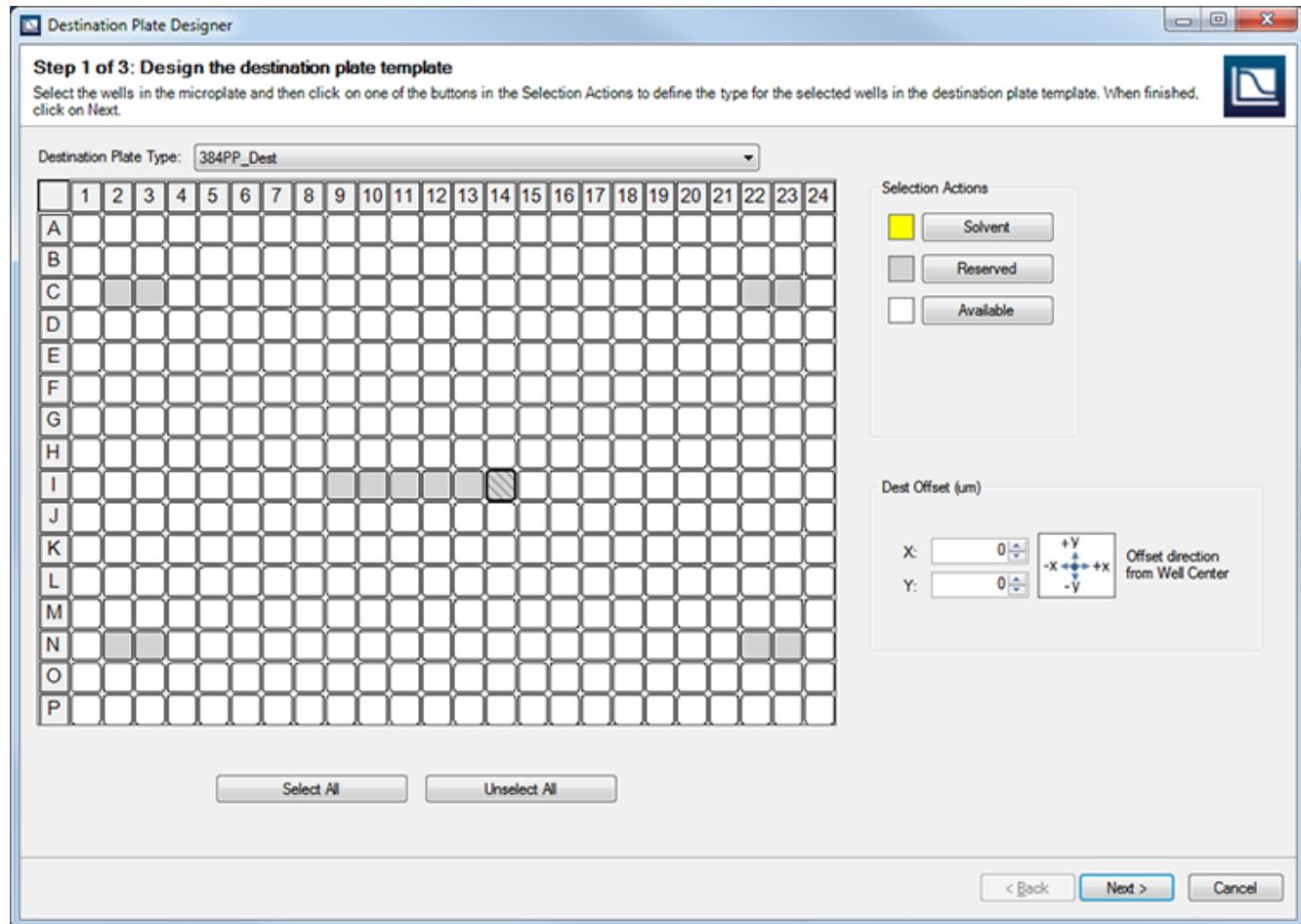
To create a destination plate:

1. Click **Add Destination** in the **Plate Template Actions** section in the **main Protocol** window to start the **Destination Plate Designer** wizard. See Figure [Plate Template Actions section](#).

The **Destination Plate Designer** wizard displays the destination plate template in a new window and guides the user through each step needed to create the destination plate design. It displays **Control** and **Solvent** buttons if controls and solvents were previously added to a source, fixed, or intermediate plate.

For example, if controls do not exist in the source or fixed plates, the **Control** button would not be shown in the dialog box below.

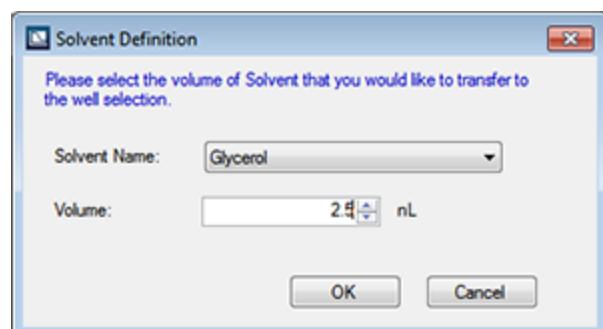
Figure 30: Destination Plate Designer dialog box - Step 1



2. In the **Destination Plate Designer** dialog box, select the **Destination Plate Type**.

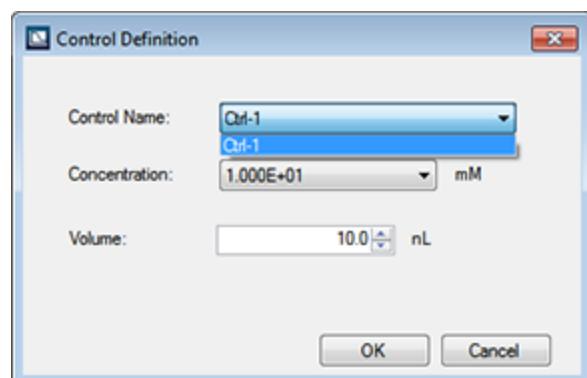
Note: Since the destination plate will be receiving the liquid transfer, a broader range of plate types is available.

3. Optionally, if you want to move where the drop should land during a transfer, set the values for **Dest Offset** for horizontal (X) value and the vertical (Y) value for the offset of the drop. A positive number indicates the offset to move to the right or up and a negative number indicates the offset to move to the left or down. The default drop location when a transfer is performed is the center of the destination well.
4. Select wells where samples are not to be dispensed to use for the solvent and click **Solvent**. This can be a negative control on the assay plate.
5. In the **Solvent Definition** dialog box, enter the **Solvent Name** and the **Volume**.

Figure 31: Solvent Definition dialog box

6. Click **OK** to close the **Solvent Definition** dialog box. The assigned wells change to yellow.
7. Select the desired wells that will be reserved and click **Reserved**.
8. Select the wells that will be used as a control and click **Control**.
9. In the **Control Definition** dialog box, select the **Control Name** from the drop-down list. The list of control names available depends on the controls previously created on the source or fixed plates. Enter the **Concentration** and the **Volume**.

Controls can be selected and diluted like samples.

Figure 32: Control Definition dialog box



Note: The default concentration unit is specified in the **Main Protocol** window. The Echo Dose-Response software default concentration unit is M, but the user can change this to any of the available concentration units. Additional concentration units can be created using the **Concentration Unit Definitions** window. For more information, see [Concentration Unit Definitions](#).

10. Click **OK** to close the **Control Definition** dialog box. The assigned wells will change to blue.
11. Click **Next** to continue to the next step to define the dose-response data points. The **Destination Plate Designer** dialog box is shown. It contains the **Table** and **Curve** tabs shown in the figures below.

Figure 33: Destination Plate Designer - Step 2 (Table tab)

#	Source Concentration (mM)	Transfer Volume (nL)	Backfill Volume (nL)	Assay Volume (μL)	Final Concentration (mM)	Final %Solvent
1	1.000E+01	250.0	0	0	1.000E+01	100.000
2	1.000E+01	80.0	170	0	3.200E+00	100.000
3	1.000E+01	25.0	225	0	1.000E+00	100.000
4	3.187E-01	247.5	2.5	0	3.155E-01	100.000
5	1.000E+01	2.5	247.5	0	1.000E-01	100.000
6	3.187E-01	25.0	225	0	3.187E-02	100.000
7	3.187E-01	7.5	242.5	0	9.560E-03	100.000
8	3.187E-01	2.5	247.5	0	3.187E-03	100.000
9	1.020E-03	245.0	5	0	9.999E-04	100.000
10	1.020E-03	77.5	172.5	0	3.163E-04	100.000

A user can copy a value or a set of values from a single cell or a set of cells from the Curve Table.

To copy data from the Curve Table:

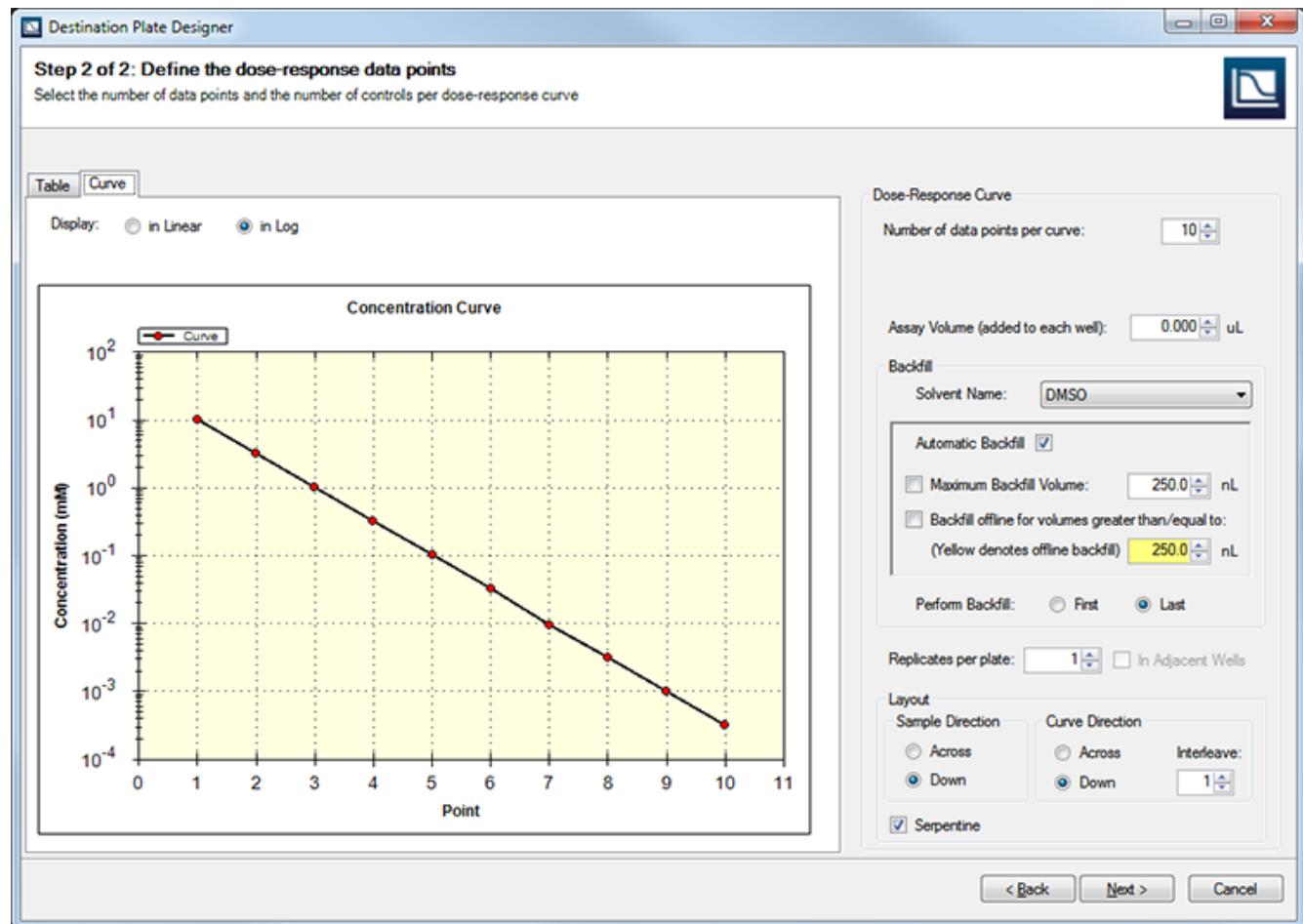
- Select a single cell or multiple cells in the Curve Table. To select multiple cells, click on a cell, drag, and release.
- Click the **Ctrl+C** key combination, or right-click and select **Copy Selected** from the context menu. When a single cell is selected, the context menu contains **Copy** and **Paste** menu options. Select the **Copy** menu item.

Once a cell or multiple cells have been copied, a user can paste a single value or a set of values. The set of values can also be copied from a column of data from Excel or any other text editor into a clipboard.

To paste data to the Curve Table:

- Click on any single cell inside the **Transfer Volume** column to turn the cell into an editable control.
- Click the **Ctrl+V** key combination, or right-click and select **Paste** from the context menu. When pasting a set of values, data is pasted starting from a selected cell and going down until the end of the cells or until all data is used from the set in the clipboard.

Figure 34: Destination Plate Designer - Step 2 (Curve tab)



- Enter the **Number of data points per curve**. The Data Point Curve table automatically updates to display the number of rows corresponding to the number of points in the dose-response curve. In this example, a 12-point curve was selected.
- If a source or fixed plate contains controls, a check box to **Include Controls in Dropdown Lists** appears under the **Number of data points per curve**. If this option is selected, the controls will be available in the **Source Concentration** drop down list for curve creation and included in the **Number of data points per curve** count.
- Enter the final **Assay Volume** for all the destination wells for the plate.
- Select the **Solvent Name**.
- Select **Automatic Backfill** to automatically back-fill each concentration point to produce a normalized curve. The Echo Dose-Response application will calculate the back-fill volumes required and coordinate the actual filling from solvent wells of a source plate, fixed plate, intermediate plate, or use of an offline bulk dispenser. This can be performed for multiple solvents. Select each solvent from the **Solvent Name** drop down list and fill in the parameters appropriately.
- Select **Backfill off-line for volumes great than/equal to** and enter the **Volume** to limit for solvent transfers performed by the Echo system. Back-fill volumes equal to or larger than this will need to be transferred by an offline system (handheld pipette, or bulk dispenser). The concentration points that require this will be highlighted in yellow.
- Select **Maximum Backfill Volume** to enter a maximum **Volume** for normalization. All concentration points with a transfer volume below this will be backfilled to this volume.

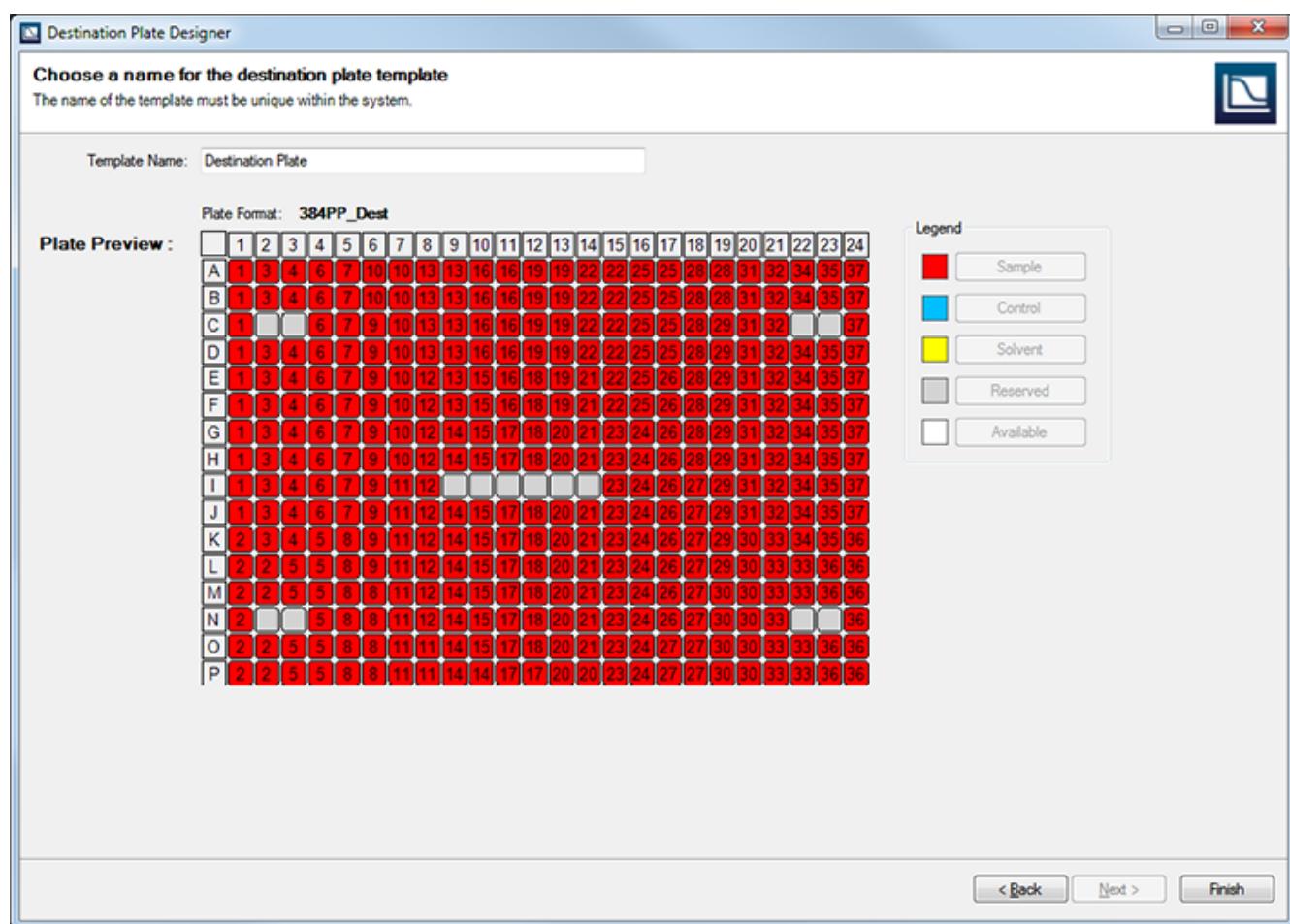
19. Choose **First** or **Last** for **Perform Backfill**. **First** schedules transfers of back-fill solvent to occur before all sample transfers. **Last** schedules transfers of back-fill solvent to occur after all sample transfers.
20. In the table, select the sample **Source Concentrations and (Control** and/or **Control Concentration[if available]**) and enter the **Transfer Volume** for each data point. The Dose-Response application calculates the **Backfill Volume** of the solvent and the **Final Concentration** for each data point and adds them to the dose-response data point curve table.
21. Enter a value for **Replicates per plate**. More than one replicate can be mapped in numeric order or grouped by the sample number by checking **In Adjacent Wells**.
22. Select the **Sample Direction** (Across or Down).
23. Select the **Curve Direction** (Across or Down) and set the value for **Interleave**.



Note: The Dose-Response application fits all the sample concentrations for a curve on the same plate if possible. If a complete curve cannot fit on the plate, it is moved to the next plate.

24. If the **Sample Direction** and **Curve Direction** match, the **Serpentine** checkbox becomes available. This layout specifies how the curve wraps to the next row (when the curve direction is Across) or to the next column (when curve direction is Down) if the curve does not align evenly with the number of wells in the row or column. When using Serpentine layout, the wrapping of the curve to the next column changes direction with every other row or column. Without Serpentine layout, the wrapping resets to the top of the next row or column. Select the **Serpentine** checkbox if desired.
25. Click **Next** to continue to the final step to enter a unique destination **Template Name**.

Figure 35: Destination Plate Designer dialog box - Step 3



26. Click **Finish** to close the Destination Plate Designer dialog box.

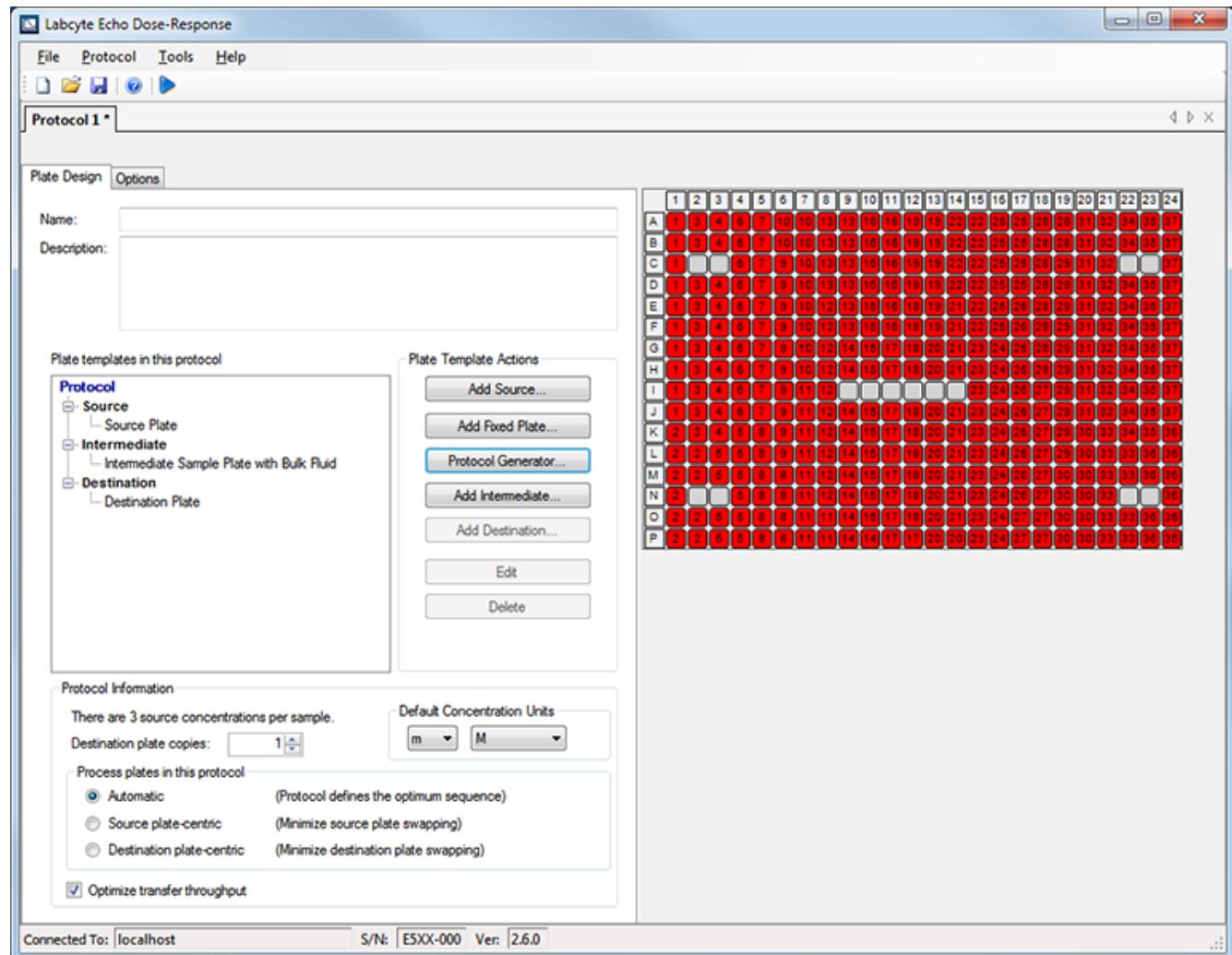
The destination plate definition is complete. The **Plate Design** tab displays the design of the destination plate.

27. To edit or delete the destination plate template, select the destination plate template in the protocol tree and click **Edit** to open the **Destination Plate Designer** dialog box or click **Delete** to remove the destination plate.
28. To save the protocol, select **Save** from the **File** menu and enter a file name in the prompt box. The protocol is saved with the file extension **.edr** in the default directory **C:\Labcyte\Echo\Protocols**.



Note: The protocol file can be saved to different directories. It is recommended to save the protocol often while working on it.

Figure 36: Destination Plate Created



Advanced Features

There are many advanced features that a user can use when creating protocols. The topics below describe what these features are and how to use them.

- [Adding a Fixed Plate](#)
- [Importing a Pick List](#)

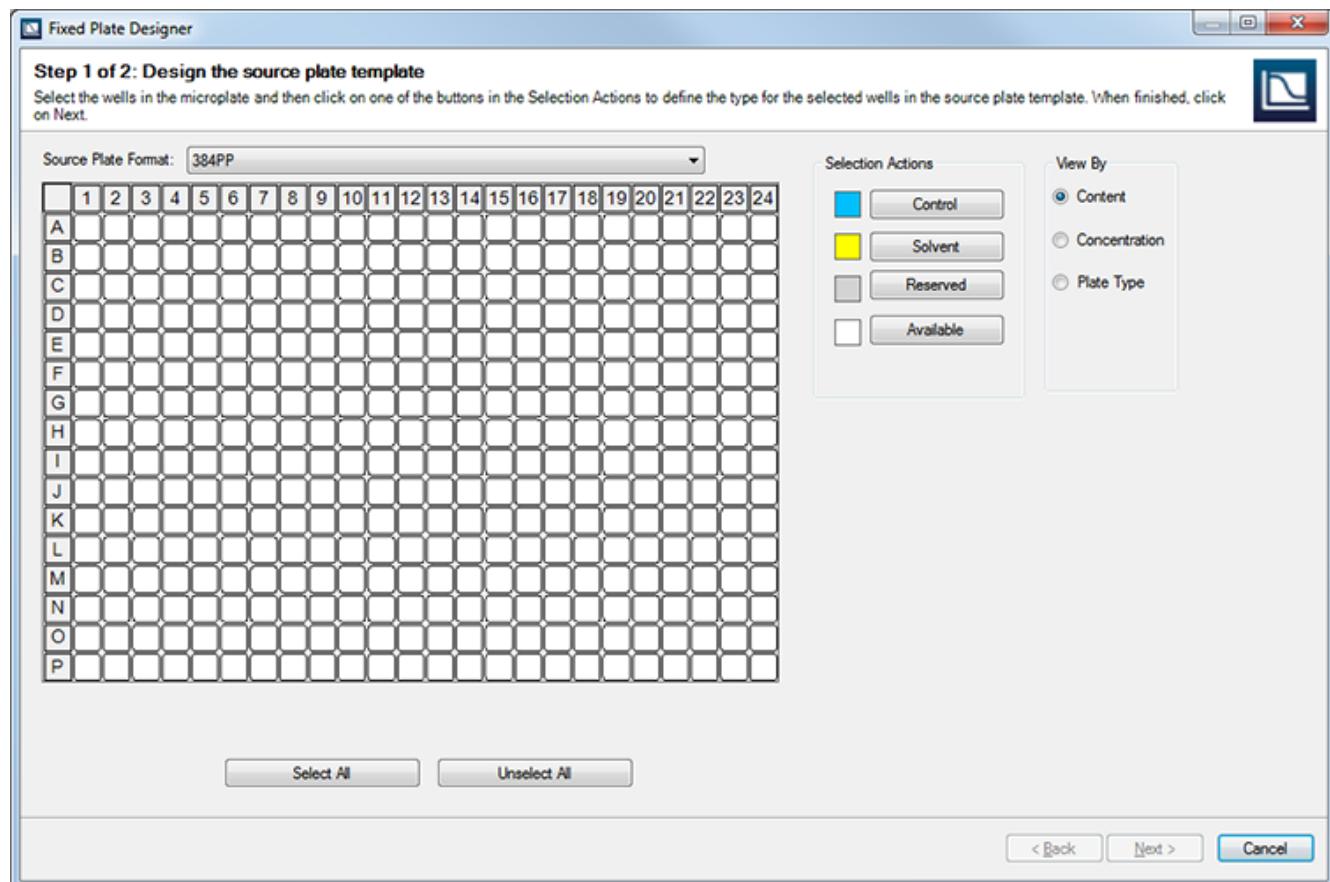
Adding a Fixed Plate

The fixed plate template defines the plate map of controls and/or solvents to be transferred to the assay plate.

To create a fixed plate:

1. Click **Add Fixed Plate** in the **Plate Template Actions** section in the **main Protocol** window to start the **Fixed Plate Designer** wizard.
- The **Fixed Plate Designer** wizard displays the fixed plate template in a new window and guides the user through each step needed to create the fixed plate design.
2. In the **Fixed Plate Designer** dialog box, select the **Source Plate Format**.

Figure 37: Fixed Plate Designer dialog box - Step 1



Source Plate Format describes the physical properties of the plate: for example, 384 wells in a polypropylene plate (384PP).



Note: The fixed plate must be an Echo qualified plate.

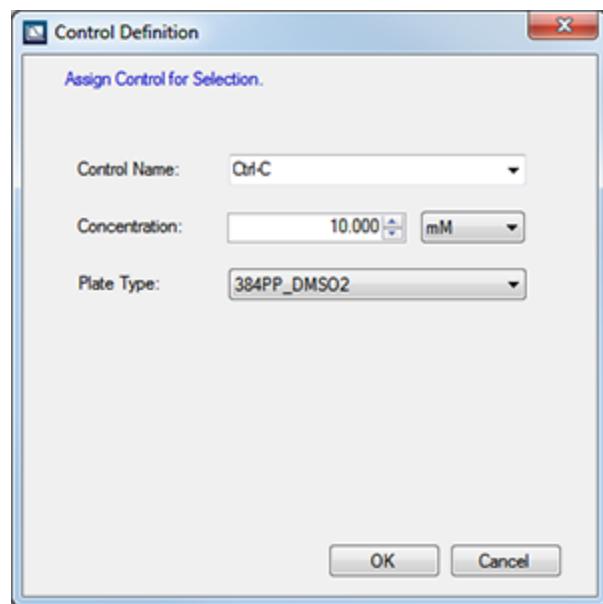
3. Select the well or well(s) that will contain the control and click the **Control** button in the **Selection Actions** section.

4. In the **Control Definition** dialog box, enter the control name, control concentration, and units. The control name is required, but the control concentration and units are optional. If a control is created with a concentration, then the control will be available for intermediate concentration.

Select the plate and fluid class from the **Plate Type** menu.

Plate Type describes the fluid type that is used by the control. Controls with different fluid types can be assigned to the same fixed plate. For more information, see the source plate in the Echo Dose-Response Example C, located at C:\Labcyte\Echo\Protocols.

Figure 38: Control Definition dialog box



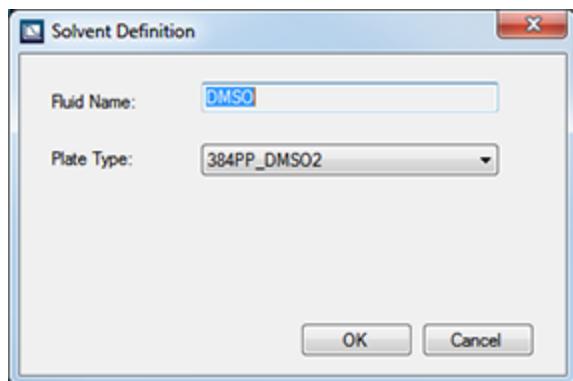
Note: The default concentration unit is specified in the **Main Protocol** window. The Echo Dose-Response software default concentration unit is M, but the user can change this to any of the available concentration units. Additional concentration units can be created using the **Concentration Unit Definitions** window. For more information, see [Concentration Unit Definitions](#).

5. Click **OK** to close the **Control Definition** dialog box. The assigned well(s) change to blue and are labeled *a*. The *a* signifies the first concentration of the control.

To view the control name and concentration, move the cursor over the assigned well. If selecting a control without a defined concentration, only the control name will appear.

6. To add another control to the plate, repeat step 3-6.
7. Select the well or well(s) that will contain the solvent and click the **Solvent** button in the **Selection Actions** section.
8. In the **Solvent Definition** dialog box, select the plate and fluid class from the **Plate Type** menu.

Plate Type describes the solvent type that is used by the sample. Different type of solvents can be assigned to the same fixed plate. For more information, see the source plate in the Echo Dose-Response Example C, located at C:\Labcyte\Echo\Protocols.

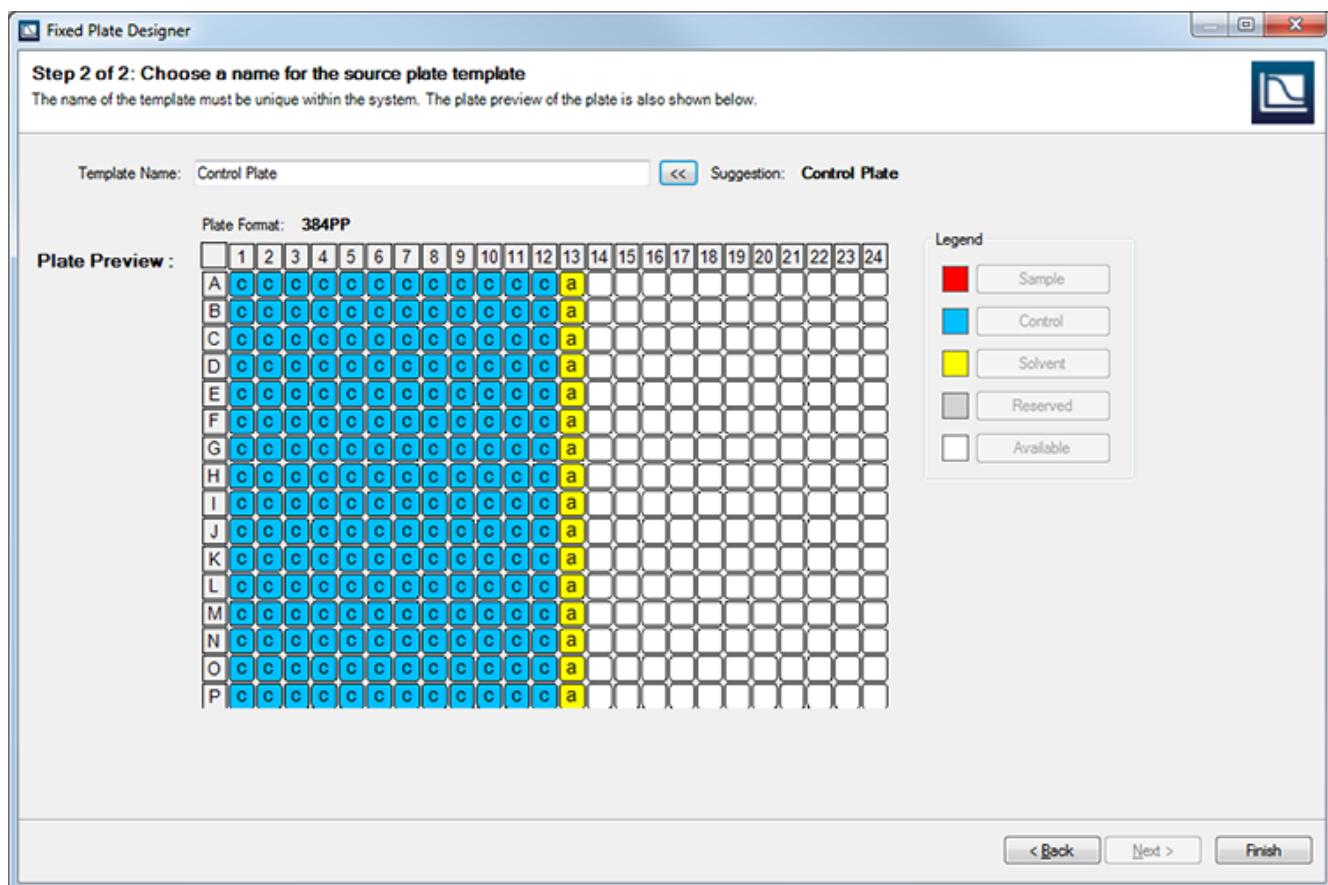
Figure 39: Solvent Definition dialog box

9. Click **OK** to close the **Solvent Definition** dialog box. The assigned well(s) change to yellow and are labeled α . The α signifies the first concentration of the sample.



Note: If the user moves the cursor over a solvent, the source well and plate type will appear.

10. To add another solvent to the plate, repeat step 7-9.
11. Click **Next** to display the next screen and enter the fixed template name. Type a name in the **Template Name** field or click the left arrow button to accept the suggested name.

Figure 40: Fixed Plate Designer dialog box - Step 2

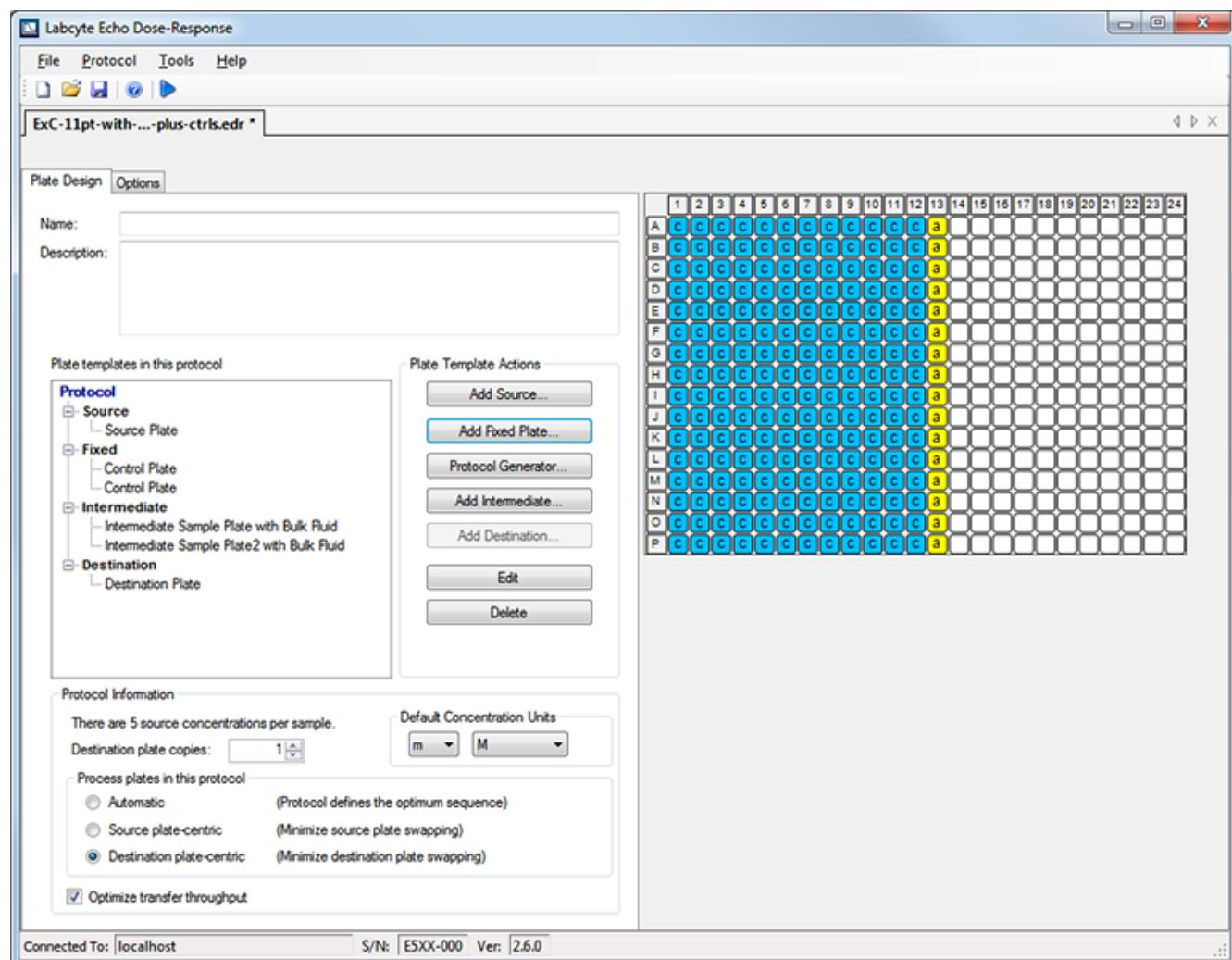
12. Click **Finish** to close the Fixed Plate Designer dialog box.

The fixed plate definition is complete. The **Plate Design** tab displays the design of the fixed plate.

13. To edit or delete the fixed plate template, select the fixed plate template in the protocol tree and click **Edit** to open the **Fixed Plate Designer** dialog box or click **Delete** to remove the fixed plate.
14. To save the protocol, select **Save** from the **File** menu and enter a file name in the prompt box. The protocol is saved with the file extension .edr in the default directory C:\Labcyte\Echo\Protocols.



Note: The protocol file can be saved to different directories. It is recommended to save the protocol often while working on it.

Figure 41: Fixed Plate Selected

Importing a Pick List

Importing a pick list from the **Run Options** dialog box is a quick way to run existing Echo Dose-Response protocols with partial source plates as defined by an input file. The pick list indicates which samples from a source plate defined in the protocol will be transferred.

 **Note:** The pick list cannot be saved in the protocol. Pick lists should specify sample transfers only. Transfers from wells with solvent or control should be omitted.

Transfers from wells with solvent or control should be omitted. The pick list is typically created in a text or spreadsheet file.

Guidelines for defining pick list:

- Pick lists using row and column location must use the one-based coordinate system, which means that the first well in the upper left corner of the microplate is labeled row 1, column 1, or (1,1).
- Pick list must be saved in .csv file format to be used by the Echo Dose-Response application.

 **Note:** If an error is encountered when importing a pick list, the error message will show the column data that is needed.

 **Note:** Pick lists can specify transfers from plates containing intermediate concentrations. These plates can be referred to in multiple pick lists to reuse an intermediate plate across multiple protocols.

Echo Dose-Response pick lists should be one of the following formats:

- Transfer location only — This pick list specifies the identification of a source plate and source well only. The sample concentrations are defined by parameters in the Echo Dose-Response protocol.
- Transfer location and concentration — This pick list specifies the identification of a source plate, source well, source concentration, source concentration units, and sample name that must be predefined in the source plate template in the protocol. This format allows each sample transfer to have a unique concentration associated with the sample. This is commonly used when stock and intermediate sample concentrations are pre-plated prior to the start of a run.

 **Note:** To match concentration values with concentration values defined in the protocol, use a scientific numerical format with 3 digits after decimal point.

To process a pick list, Echo Dose-Response requires information for the following parameters to be provided in separate columns of the pick list file:

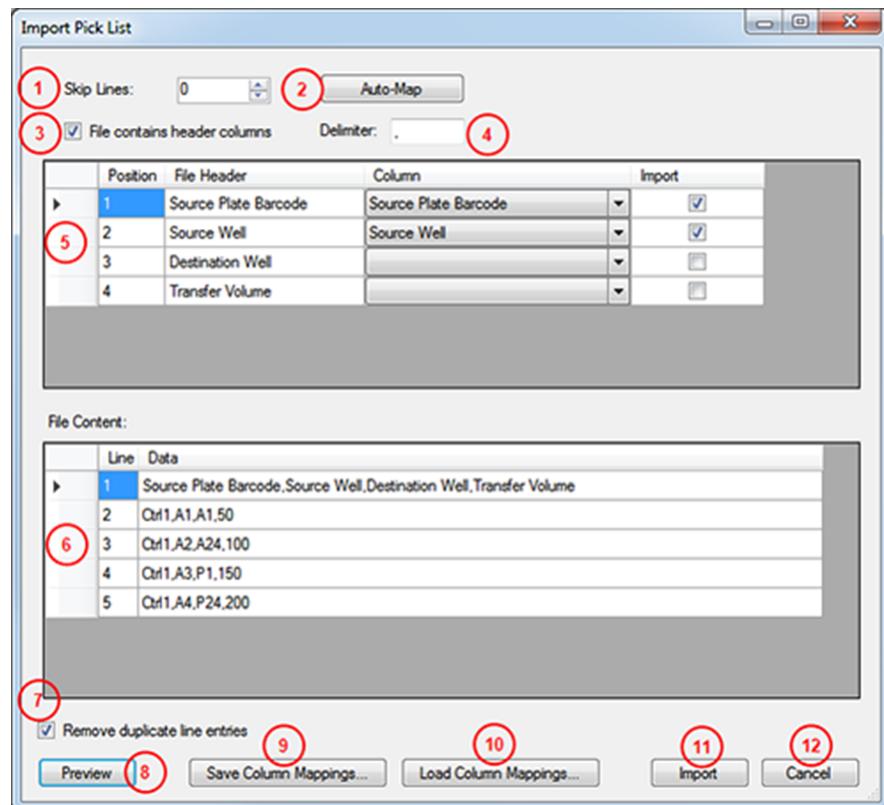
- Source Plate Name and/or Source Plate Barcode
- Source Well and/or (Source Row + Source Column)

Optionally, additional information can be supplied for the following parameters:

- Source Concentration
- Source Concentration Units
- Sample ID
- Sample Name
- Sample Group
- Sample Comment

To import a pick list:

1. To open the **Import Pick List** dialog box, select **Run** from the **Protocol** menu or click the **Run** icon in the **Toolbar**. See Figure [Run Options dialog box](#).
2. Click **Partial Plates** to pick specific plates. When the **Partial Plates** option is selected, an **Import** button is displayed to import a .csv pick list indicating the wells to be transferred.
3. Click **Import** to import the pick list.

Figure 42: Import Pick List dialog box

The table below describes the buttons/fields or sections in the **Import Pick List** dialog box and their functionality.

Table 3: Callout table for Import Pick List dialog box

Callout Number	Name	Description
1	Skip Lines	The pick list can contain heading information (for example, assay information, date, etc.), but it should be skipped when importing. Select a numerical value for Skip Lines to skip the number of specified heading lines for the column headings (header line).
2	Auto-Map button	Maps the column headings and data automatically.
3	File contains header columns	If the pick list contains a header line (column headings), select this option.
4	Delimiter	The delimiter used to separate data in the pick list file.
5	Column Details	<ul style="list-style-type: none"> • Position — Specifies the position in the file. • File Header — Specifies the header for the column. • Column — Specifies the column name. This drop-down list defines the columns that can be added to the pick list. • Import — Specifies if the data for the associated column should be imported or not.
6	Preview Result or File Content table	The results for this table are toggled based on if the user clicks the Preview or the File content button.
7	Remove duplicate line entries checkbox	Check this to remove duplicate entries in the source file when creating the pick list.
8	Preview or File content button	Toggles between the preview result showing the IDs, corresponding source wells, and other data available and the file content result showing the line number and corresponding data.
9	Save Column Mapping to File button	Exports the column mappings to a text file.
10	Load Column Mapping from File button	Imports the column mappings to be viewed.
11	Import button	 Note: By clicking Import , the existing protocol data is overwritten and cannot be undone unless the protocol was previously saved.
12	Cancel button	Closes the Import Pick List dialog box without saving any changes.

Editing the Protocol

There are two ways to edit an existing protocol.

1. Select **Open** from the **File** menu and select the protocol to be edited.

2. Click the **Open**  icon in the **Toolbar** and select the protocol to be edited.

Once the protocol to be edited has been loaded into the **main Protocol** window, the user can add source, intermediate, fixed or destination plates, delete an existing plate, or edit the settings entered when the protocol was first created.

To edit the protocol:

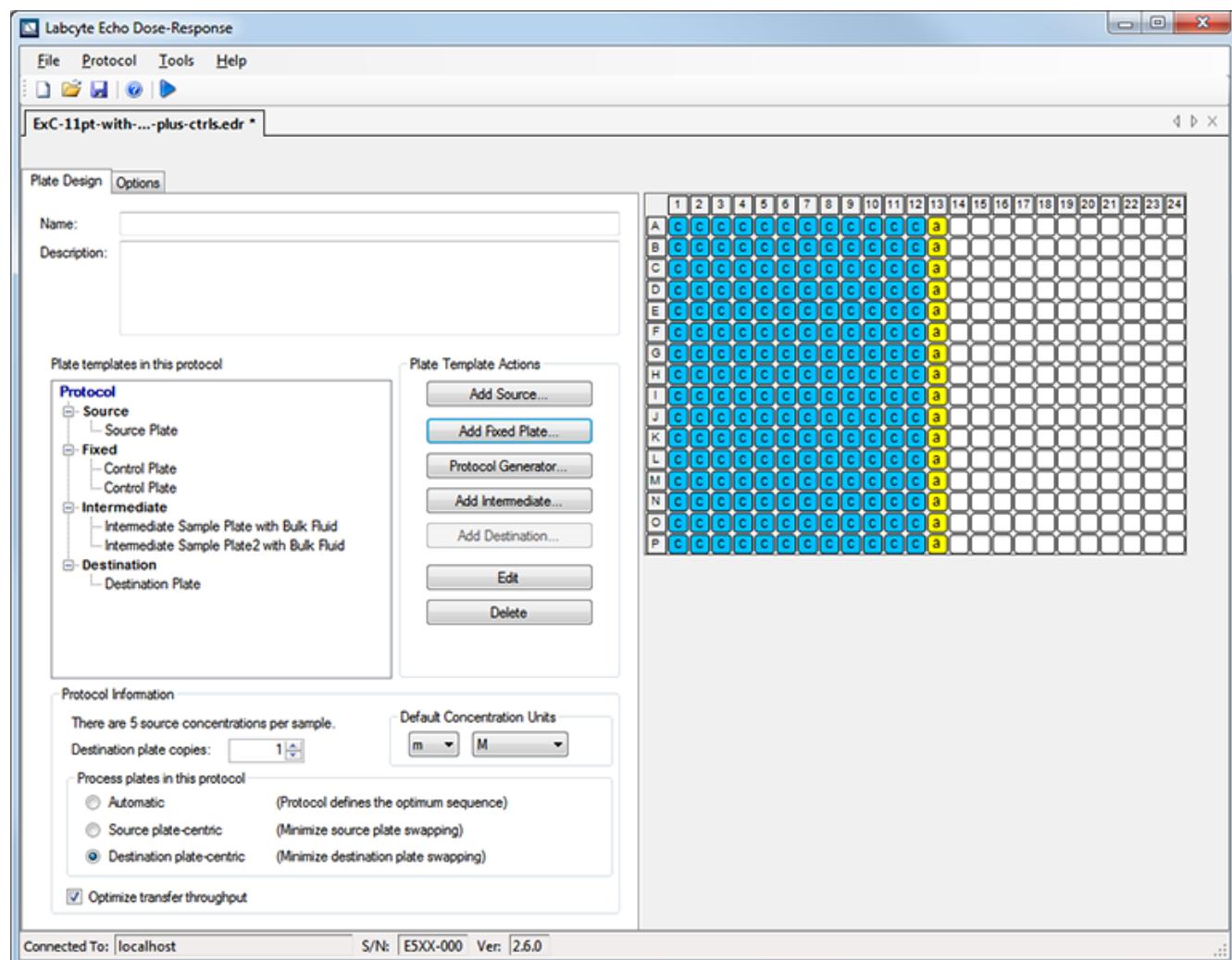
3. Select an existing plate template and click **Edit** to edit the plate or click **Delete** to delete the plate.
4. Click **Add Source** in the **Plate Template Actions** section to add a source plate. For more information, see [Creating a Source Plate](#).
5. Click **Add Intermediate** in the **Plate Template Actions** section to add an intermediate plate. For more information, see [Manually Creating Intermediate Plates](#).

6. Click **Add Destination** in the **Plate Template Actions** section to add a destination plate. For more information, see [Manually Creating a Destination \(assay\) Plate](#).



Note: This option is only available if a source plate has already been created. In addition, only one destination plate can exist per protocol. Before adding a new destination plate, the previous destination plate must be deleted otherwise this option is disabled.

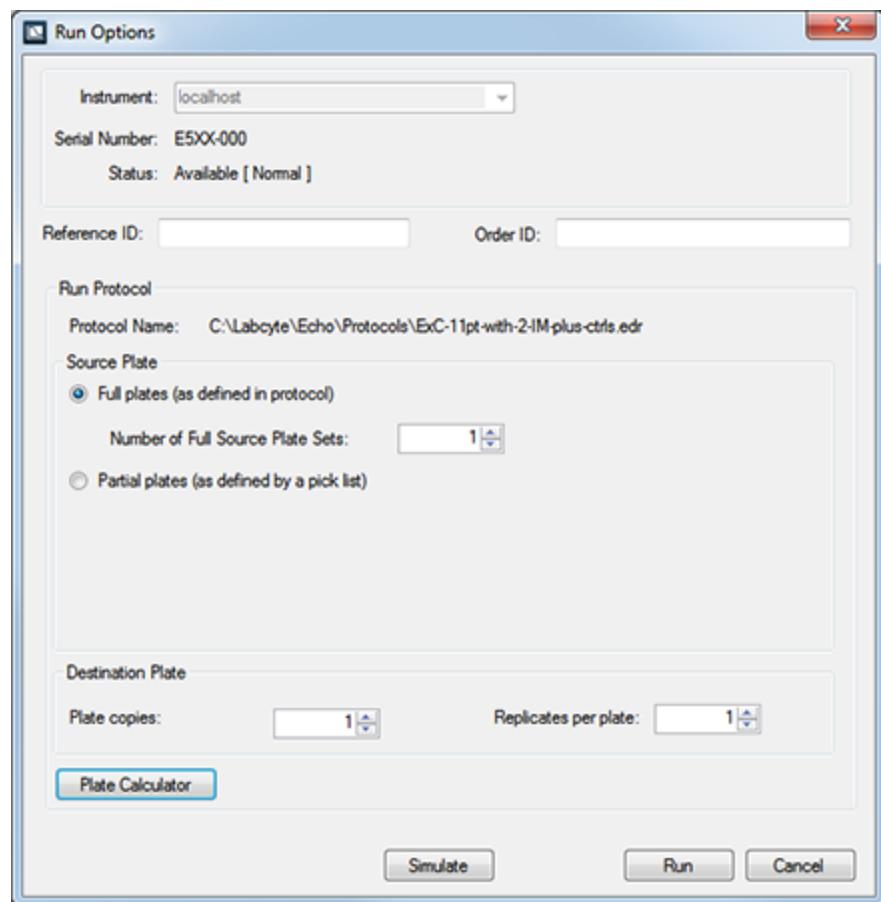
7. Click **Add Fixed Plate** in the **Plate Template Actions** section to add a fixed plate. For more information, see [Adding a Fixed Plate](#).
8. Select a previously created source plate, fixed plate, intermediate plate, or destination plate and click **Edit** to edit the plate.
9. Enter the protocol **Name** and **Description** in the **main Protocol** window.
10. In the **Protocol Information** box, enter the value for **Destination plate copies** and optionally, set the **Default Concentration Units**. For more information, see [Tools Menu](#) and [Concentration Unit Definitions](#).
11. Select one of the following choices in the **Process plates in this protocol** box:
 - **Automatic** — Protocol defines the optimum sequence.
 - **Source plate-centric** — Minimizes source plate swapping.
 - **Destination plate-centric** — Minimizes destination plate swapping.
12. Check **Optimize transfer throughput** to cause the Echo to override the order of operations specified in the protocol and reorder transfers to minimize stage and transducer movements.

Figure 43: Main Protocol window

Running a Protocol

To begin a protocol simulation or live run:

Figure 44: Run Options dialog box

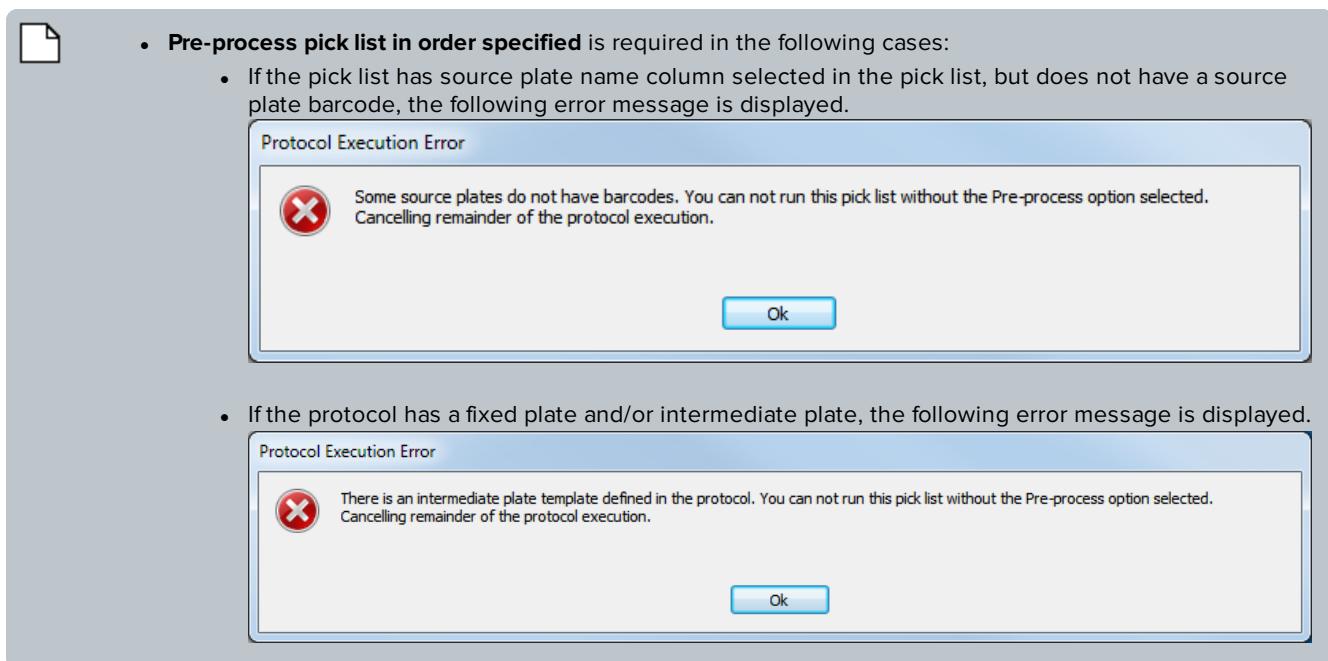
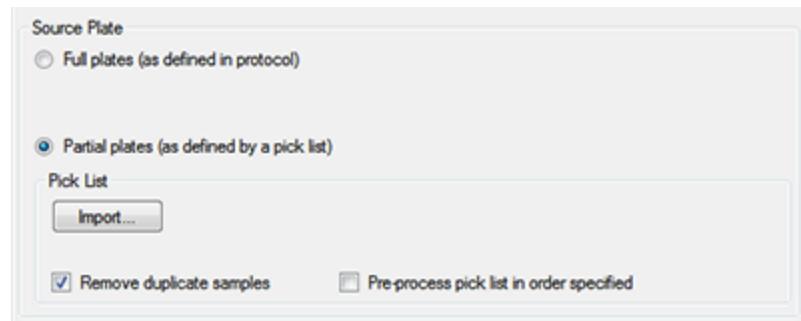


1. Select **Run** from the **Protocol** menu or click the **Run** icon in the **Toolbar**.
2. Select the **Instrument** if it is not already selected.
3. Optionally, enter a **Reference ID** and/or **Order ID** to be used to reference the run in the output report files.
4. For the **Full Plates** option defined by the protocol, specify the number of source plates to process. The number of source plates are defined in the protocol and cannot be overridden in the **Run Options** dialog box.
5. Click **Partial Plates** to pick specific plates. When the **Partial Plates** option is selected, an **Import** button is displayed to import a .csv pick list indicating the wells to be transferred. For more information on how to define a pick list, see [Importing a Pick List](#).



Note: If the **Partial Plates** option is selected, the **Remove duplicate samples** and **Pre-process pick list in order specified** options become available.

- Select **Remove duplicate samples** to eliminate redundant samples found in the imported pick list. This may occur in cases where an output file has been imported as an input file. For example, when importing the Exceptions Report as a pick list to retry missed transfers.
- Select **Pre-process pick list in order specified** to automatically optimize the order of transfers to destination plates before the run starts. This is only recommended for use with plates that can be accessed in random-access mode. If plates must be accessed sequentially, it is recommended to uncheck **Pre-process pick list in order specified** since the order of plates in the stack is fixed.

**Figure 45: Run Options dialog box**

- In the **Destination Plate** section, change the value for **Plate copies** to override the number of plate copies for the protocol. Change the **Replicates per plate** value to override the destination plate copy setting in the protocol for the replicates per plate.
- Click the **Plate Calculator** button to view the number of plates needed to run the protocol.

Figure 46: Plates Used dialog box

Plate	Number
Source Plate	2
Fixed Plate	1
Destination Plate	1
Intermediate Sample Plate with Bulk Fluid(1536LDV_DMSO)	1
Intermediate Sample Plate2 with Bulk Fluid(1536LDV_DMSO)	1

OK

8. Optionally, click **Simulate** to test the run.



Note: Running a simulation is recommended to verify the transfers defined in the protocol before a live run.

9. Click **Run** to execute the protocol.

Viewing Reports

At the end of the transfer run, the Echo Dose-Response application builds the transfer reports and stores them in a dated folder in the following location:

C:\Labcyte\Echo\Reports\Labcyte Echo Dose-Response\2018-01-30

Figure 47: Transfer Report Directory

Name	Date modified	Type	Size
_Survey_Intermediate Sample Plate with Bulk Fluid[1].csv	1/30/2018 3:12 PM	Microsoft Excel C...	1 KB
_Survey_Source Plate[1].csv	1/30/2018 10:03 AM	Microsoft Excel C...	1 KB
_Transfer_0.csv	1/30/2018 10:03 AM	Microsoft Excel C...	1 KB
_Transfer_0-01.csv	1/30/2018 3:12 PM	Microsoft Excel C...	1 KB
ESXX-000_Survey_Source Plate[1](UnknownBarcode).csv	1/30/2018 3:12 PM	Microsoft Excel C...	11 KB



Note: In the screenshot above, the date is 2018-01-30, but the date of the folder is changed depending on when the report is run. In cases where multiple reports are run on different days, multiple folders are created accordingly.

The user can open and view any of these reports in a viewer capable of viewing .csv files.

The automatically generated reports include:

- Source Plate Survey Reports
- Intermediate Plate Survey Reports
- Fixed Plate Survey Reports
- Transfer Reports

The default format for these reports can be modified by changing the layout in the **Protocol Options** tab. The location of the report is set by the **Directory** template defined in the **Protocol Options Tab** and the filename is set by the **File** template in the **Protocol Options Tab**. For more information, see [Protocol Options Tab](#).

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CHAPTER 5 | Software Reference

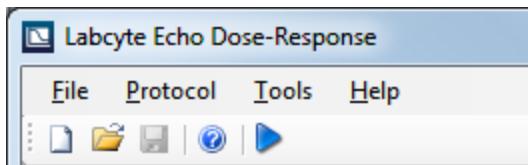
This chapter describes all of the Echo Dose-Response application screens. It includes the following topics.

- [Toolbar](#)
- [Protocol Tab](#)
- [Protocol Options Tab](#)
- [Preferences](#)
- [Labware Definitions](#)
- [Concentration Unit Definitions](#)
- [Run Protocol](#)
- [Simulator Window](#)
- [Run Status Window](#)

Toolbar

The **Toolbar** contains the **File**, **Protocol**, **Tools**, and **Help** menus, and individual icons for frequently used functions.

Figure 48: Toolbar

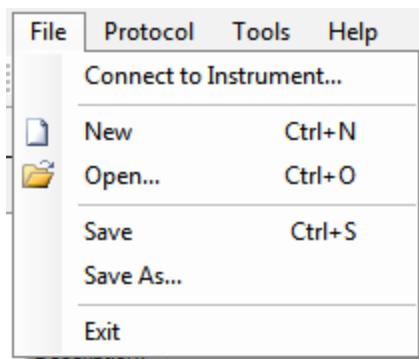


The topics below describe the functions that can be performed using the **Toolbar**.

- [File Menu](#)
- [Protocol Menu](#)
- [Tools Menu](#)
- [Help Menu](#)

File Menu

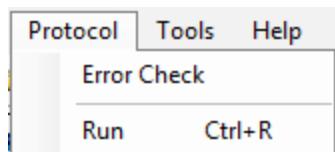
Figure 49: File menu



- **Connect to instrument** — Selects an Echo instrument to connect to the software.
- **New** — Creates a new transfer protocol.
- **Open** — Opens an existing transfer protocol.
- **Save** — Saves the current transfer protocol.
- **Save As** — Copies the current transfer protocol to a different file name.
- **Exit** — Closes the Echo Dose-Response software.

Protocol Menu

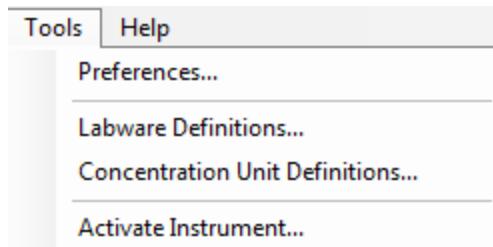
Figure 50: Protocol menu



- **Error Check** — Checks the protocol for inconsistent information.
- **Run** — Executes the transfer protocol that is displayed.

Tools Menu

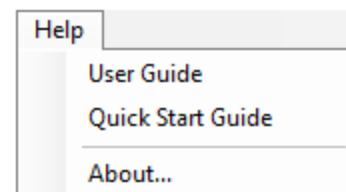
Figure 51: Tools menu



- **Preferences** — Used to set survey, output, and report options for all protocols.
- **Labware Definitions** — Manages existing labware definitions or adds new definitions.
- **Concentration Unit Definitions** — Used to create custom concentration unit definitions.
- **Activate Instrument** — Used to enter the software license key to use the Echo Dose-Response software.

Help Menu

Figure 52: Help menu

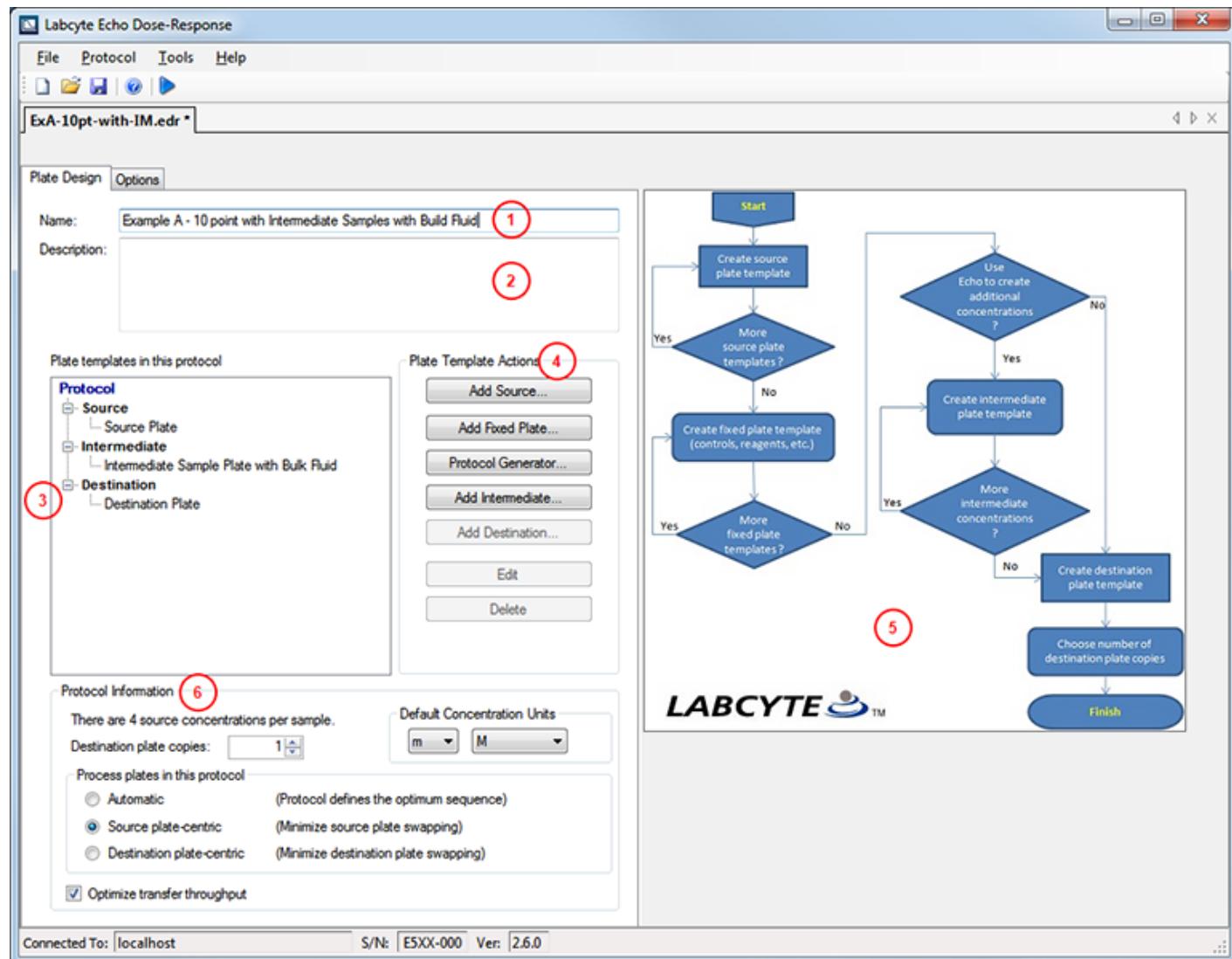


- **User Guide** — Launches the application User Guide in PDF format.
- **Quick Start Guide** — Launches the application Quick Start Guide in PDF format.
- **About...** — Display the version number of the Echo Dose-Response software.

Protocol Tab

The **Protocol** tab displays the following information:

Figure 53: Protocol tab



The table below describes the buttons/fields or sections in the **Protocol tab** and their functionality.

Table 4: Callout table for Protocol tab

Callout Number	Name	Description
1	Name	Defines the name of the protocol.
2	Description	Sets a brief description for the protocol. Information such as the list of samples that are being transferred, concentrations, number of points on the dose-response curve, and controls and backfill, if they are used can be described here.
3	Protocol Tree Container	<p>Acts as a container for the source, intermediate, destination and fixed plates created by the user.</p> <ul style="list-style-type: none"> • Source Plate Container — Contains the source plates used in the protocol. <ul style="list-style-type: none"> • Source Plate(s) — Defines the source plate(s) used in the protocol. • Intermediate Plate Container — Defines the intermediate plates used in the protocol. <ul style="list-style-type: none"> • Intermediate Plate(s) — Defines the intermediate plate(s) used in the protocol. • Destination Plate Container — Defines the destination plates used in the protocol. <ul style="list-style-type: none"> • Destination Plate(s) — Defines the destination plate(s) for the protocol. • Fixed Plate Container — Defines the fixed plate(s) used in the protocol. <ul style="list-style-type: none"> • Fixed Plate(s) — Defines the fixed plate(s) for the protocol.
4	Plate Template Actions	<ul style="list-style-type: none"> • Add Source — Opens the Source Plate Designer dialog box to create the source plate template and add it to the Protocol tree. The source plate can hold samples, controls, and solvent. • Add Fixed Plate — Opens the Fixed Plate Designer dialog box to create the fixed plate and add it to the Protocol tree. The fixed plate holds controls and solvents. • Add Intermediate — Opens the Intermediate Plate Designer dialog box to create the intermediate plate template and add it to the protocol tree. The intermediate plate can hold bulk filling reagent and sets up additional sample and control concentrations to expand the dose-response curve. • Protocol Generator — Opens the Protocol Generator Wizard dialog box with the source plate sample and control concentrations defined when the source plate was created. The wizard guides the user through each step needed to define the intermediate plate generation parameters and destination curve parameters. • Add Destination — Opens the Destination Plate Designer dialog box to create the destination plate template and add it to the protocol tree. • Edit — Opens the selected plate in the protocol tree for editing. • Delete — Deletes the selected plate in the protocol tree.
5	Workflow and Template preview	The workflow diagram is displayed whenever a protocol is open. It serves as a reference tool for planning the protocol. When the user selects any template in the template tree (left pane), the plate design for the template is displayed in the right pane.
6	Protocol Information	<p>Used to set protocol options that apply to the entire protocol.</p> <ul style="list-style-type: none"> • Destination plate copies — Number of times the plate should be copied. • Default Concentration Units — The default concentration units to be used when creating a plate. If the user does not change this value, the default concentration unit is M. • Process plates in this protocol — Defines the order in which to process the plates. <ul style="list-style-type: none"> • Automatic — Protocol defines the optimum sequence. (default) • Source plate-centric — Minimizes source plate swapping. • Destination plate-centric — Minimizes destination plate swapping. • Optimize transfer throughput — Echo overrides the order of operation specified in the protocol and reorder transfers to minimize stage and transducer movements.

Error Check

The **Error Check** command allows the user to check the protocol for inconsistent information.

To check the current protocol for errors, in the **main Protocol** window, select the **Error Check** menu option.

Run

The **Run** command initiates the transfer protocol that is displayed.

To run the current transfer protocol, in the **main Protocol** window, click the **Run**  icon or select the **Protocol > Run** menu option. For more information, see [Run Protocol](#).

Protocol Options Tab

The **Protocol/Options** window is accessible by clicking the **Options** tab behind the **Protocol** tab. This window enables the user to customize the survey, output, and reporting options for the Echo Dose-Response application. These settings are automatically used for the protocol created.

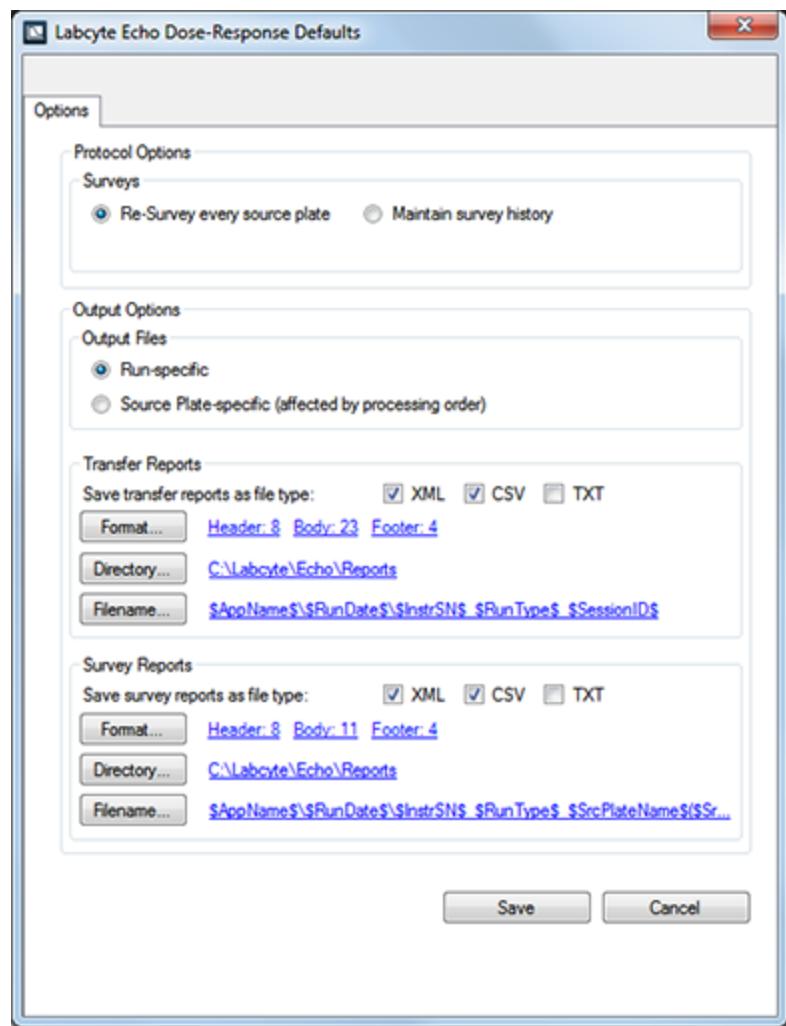
Each of the sections in the **Protocol Options Tab** is explained in the following topics.

- [Understanding Protocol Options](#)
- [Understanding Output Options](#)

Understanding Protocol Options

The **Protocol/Options** window displays the following protocol and output options.

Figure 54: Options tab



Note: The options settings are similar to the **Preferences/Options** window, but apply only to the protocol displayed.

Surveys

There are two ways to manage survey history:

- **Re-Survey every source plate** — Default setting that automatically surveys the source plate every time the protocol is run. Survey history is saved, but not referenced each time.
- **Maintain survey history** — Used to store survey data for source plates.

With **Maintain survey history** chosen, information from the initial survey is referenced for subsequent transfers — eliminating the requirement to resurvey.

This feature can reduce processing time during a protocol run — particularly if the source plate is swapped several times during a protocol run. The user can specify the length of time that the survey data is retained, from a few minutes to never resurvey.

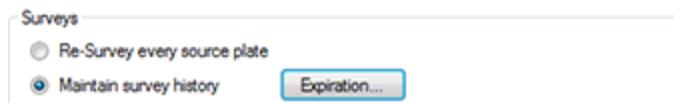
The software stores the survey data by plate barcode; therefore, the data is independent of the protocol and can be used across different protocols until the expiration time.



Note: Various factors can affect the content of the source wells, such as DMSO concentration, humidity, and exposure time during the transfer run; therefore, consider these factors when selecting an expiration time. For additional information, read the user note “*How Long Will A Survey Last When Performing Acoustic Droplet Ejections?*” on <http://www.labcyte.com> (Library > Resources > Notes).

The **Maintain survey history** function is located in the **Options** tab.

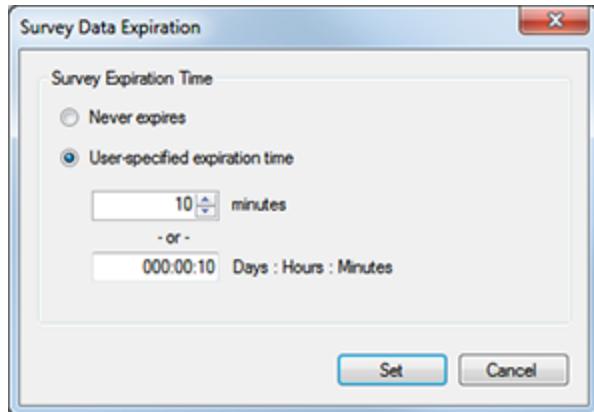
Figure 55: Surveys box



If the user selects **Maintain survey history**, an **Expiration** button appears.

Click **Expiration** to open the **Survey Data Expiration** dialog box and set the expiration values.

Figure 56: Survey Data Expiration dialog box



The following parameters can be set in the **Survey Data Expiration** dialog box:

- **Never expires** — Existing survey data is always re-used.
- **User-specified expiration time** — Existing survey data is re-used for the time that is specified in **minutes** or **Days:Hours:Minutes**.



Note: If a stored survey does not cover the plate area that is required by the protocol (for example, some of the wells were empty when the plate was originally surveyed, but then filled later on), the software will survey the new wells and add this data to the stored survey data.

The stored survey data is instrument-specific. For example, if a source plate is surveyed in instrument A, the stored survey data cannot be used in instrument B.

Although survey history can be used indefinitely, it is not recommended.

Understanding Output Options

The output options are explained in the following topics:

- [Output Files](#)
- [Transfer and Survey Reports](#)

Output Files

The **Output Files** box in the **Options** window organizes the survey and transfer results according to the following criteria:

Figure 57: Output Files options



The following parameters can be set for output files:

- **Run-specific** — All protocol data is stored in one file, in the order that the results are generated.
- **Plate-specific** — Protocol data is stored in separate files, based on source or destination plates, depending on the process order specified in the **main Protocol** window.

Transfer and Survey Reports

File Type

Survey and transfer results can be saved to the following file formats that are compatible with most laboratory information systems:

- **XML** (extensible markup language)
- **CSV** (comma separated values)
- **TXT** (text file)

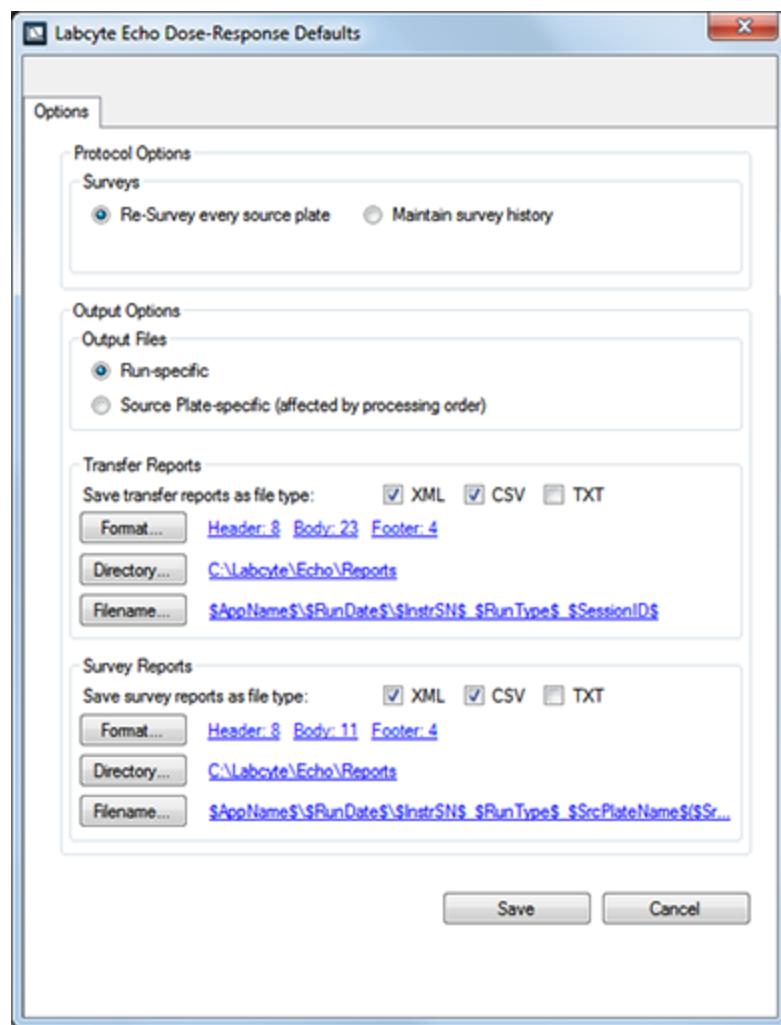
The results are saved in the formats selected, and stored in the directory specified in the **Directory** option.

Report Format

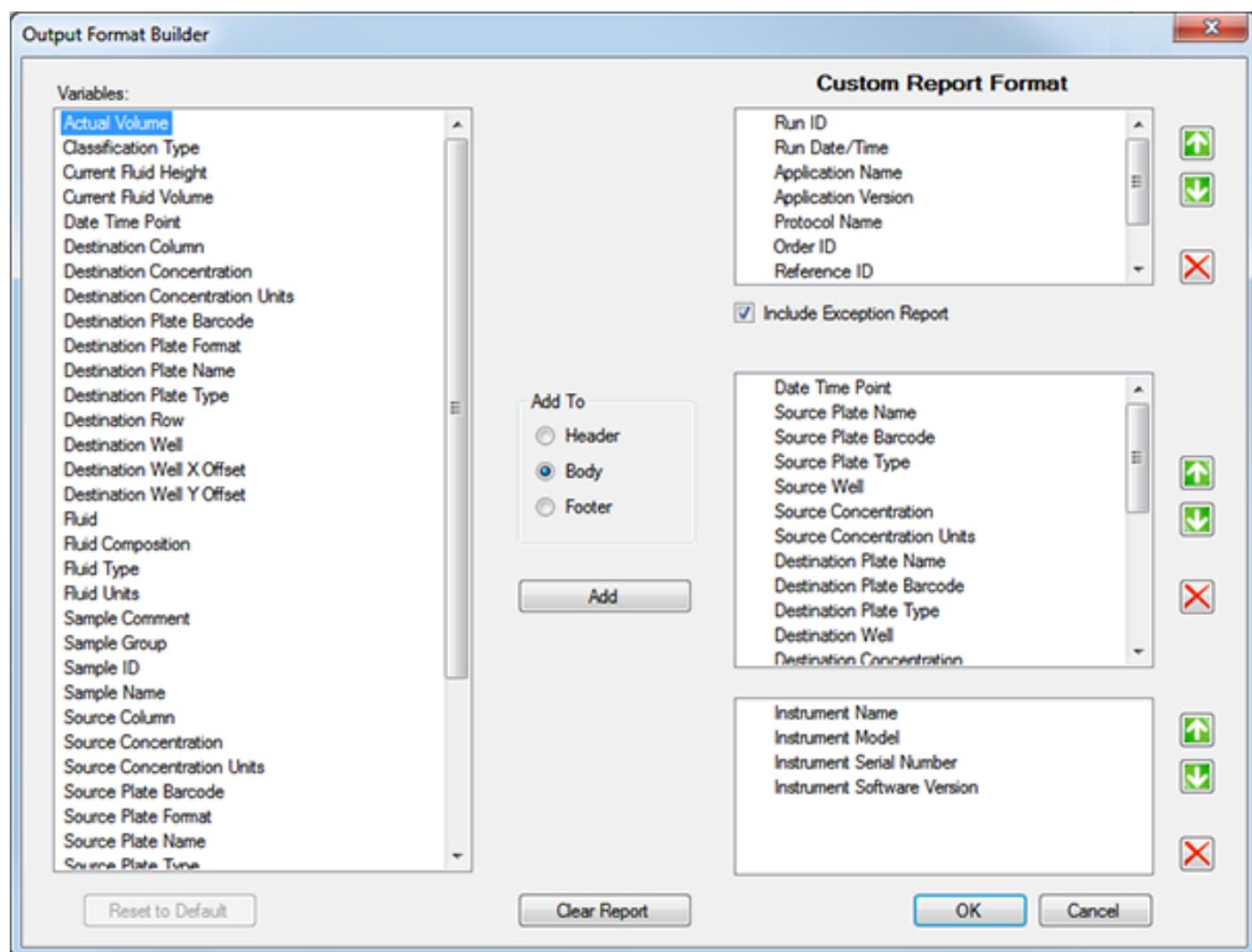
The survey and transfer reports can be customized by clicking the **Format** button in the **Protocol Options** tab.



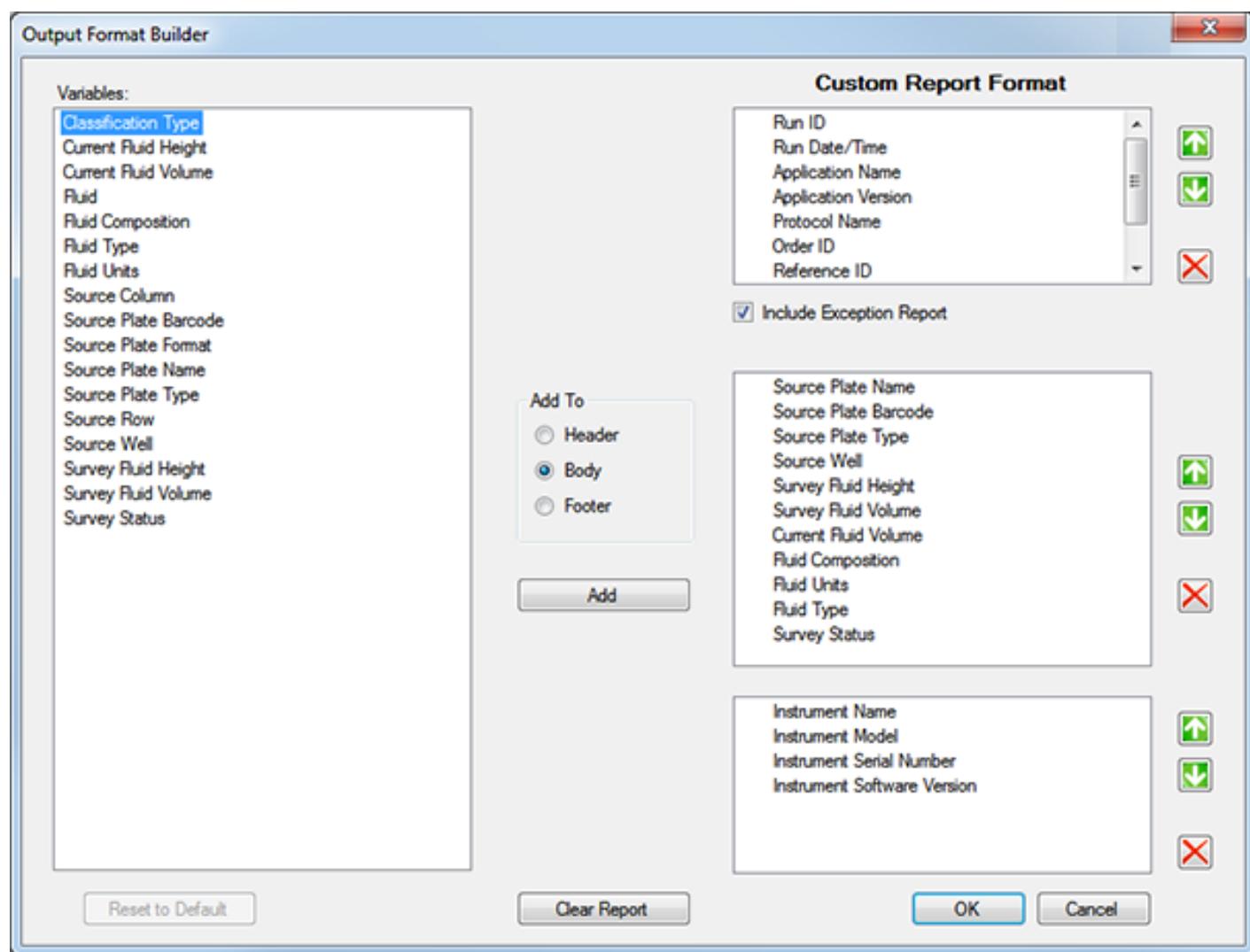
Note: The **DEFAULT** settings in the **Transfer and Survey Reports** are from the **Preferences/Options** window.

Figure 58: Report Format Options

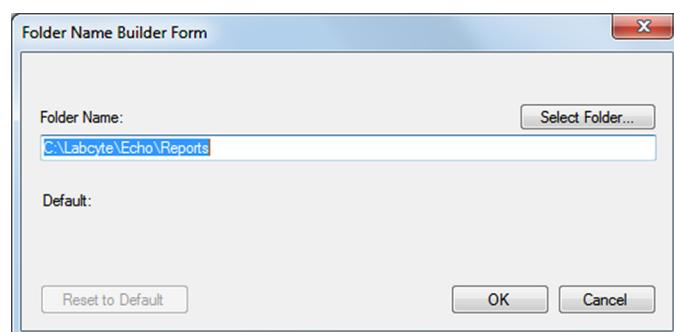
The **Output Format Builder** dialog box shows all the report variables (left selection box) that can be added to the report format (right selection boxes). The list of variables changes with the report area selected.

Figure 59: Transfer Output Format Builder

Note: The **Include Exception Report** option parses out any transfers that were not initiated because of a failed survey and promotes them to the top of the output file.

Figure 60: Survey Output Format Builder**Report Directory**

The default report directory for both surveys and transfers is C:\Labcyte\Echo\Reports. A different directory can be specified by clicking the **Directory** button and entering a new location. Use the **Select Folder** button to browse to a different directory.

Figure 61: Folder Name dialog box

Report Filename

The Echo Dose-Response application uses the following default formats to report the survey and transfer results:

Survey results file name —

`$AppName$\$RunDate$\$InstrSN$_$RunType$_$SrcPlateName$ ($SrcPlateBarcode$)`

For example:

Labcyte Echo Software\2018-02-05\E5XX-0001_Transfer_Plate1 (1-989992-0)

Transfer results file name —

`$AppName$\$RunDate$\$InstrSN$_$RunType$_$SessionID$`

For example:

Labcyte Echo Software\2018-02-05\E5XX-0001_Transfer_123

The survey and transfer formats can be changed by clicking the **Format** button in the **Protocol Options** tab.

Figure 62: Transfer Filename Builder

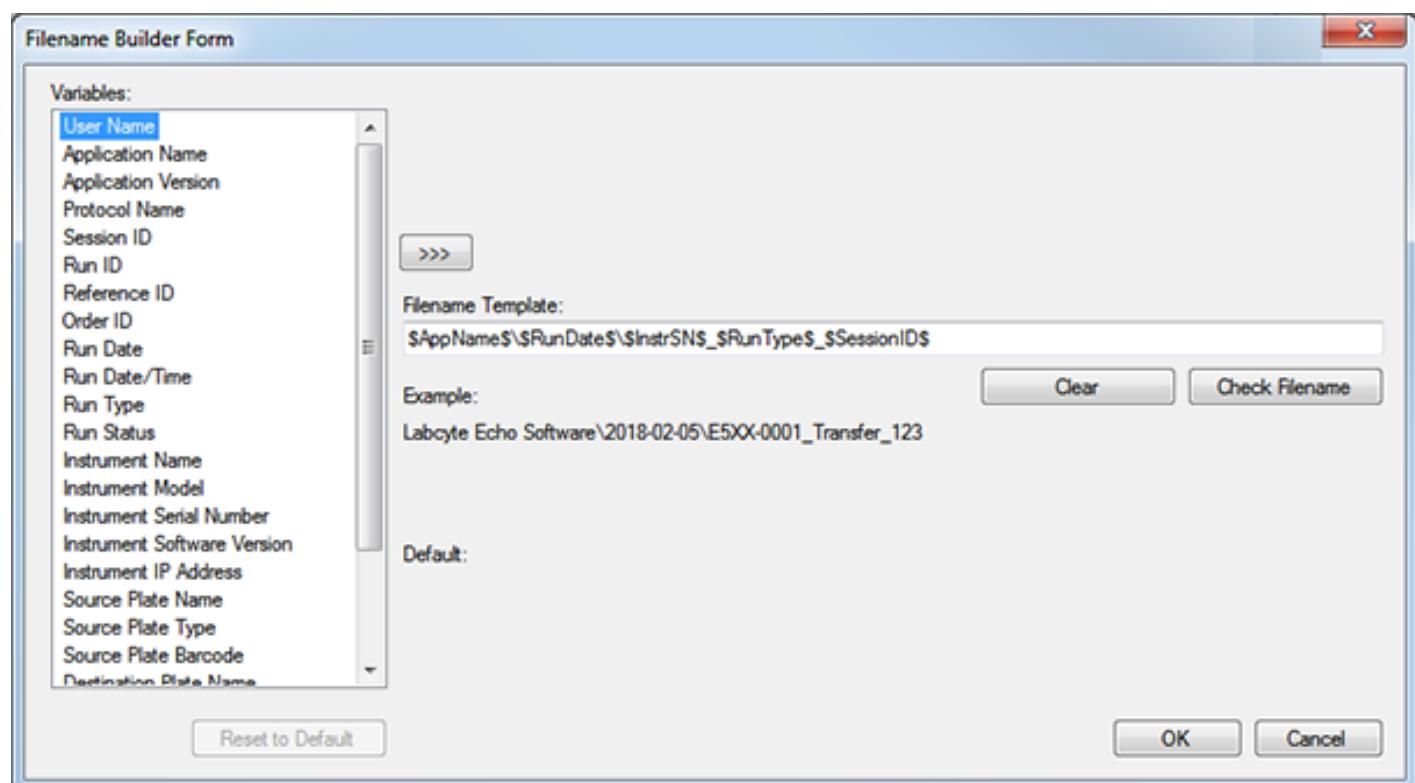
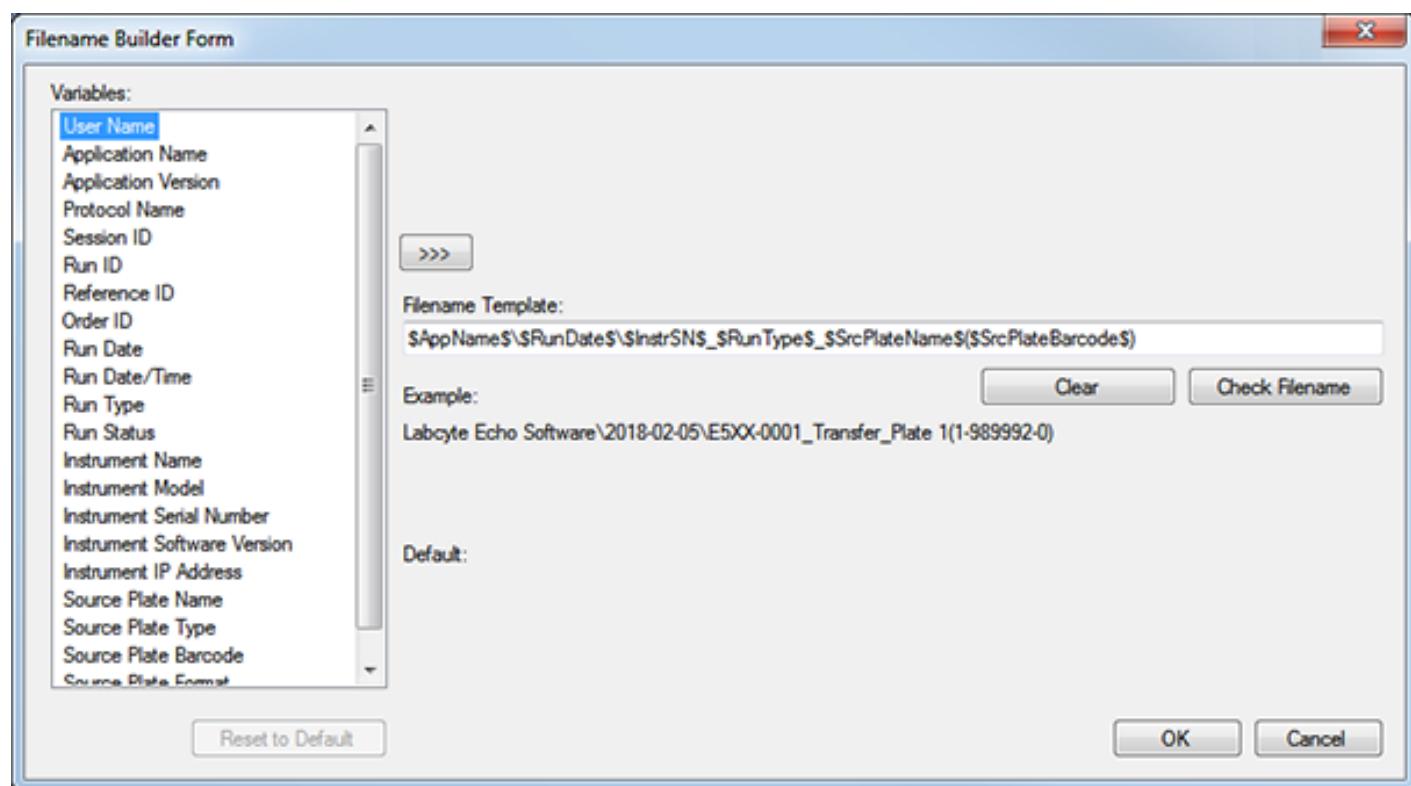


Figure 63: Survey Filename Builder

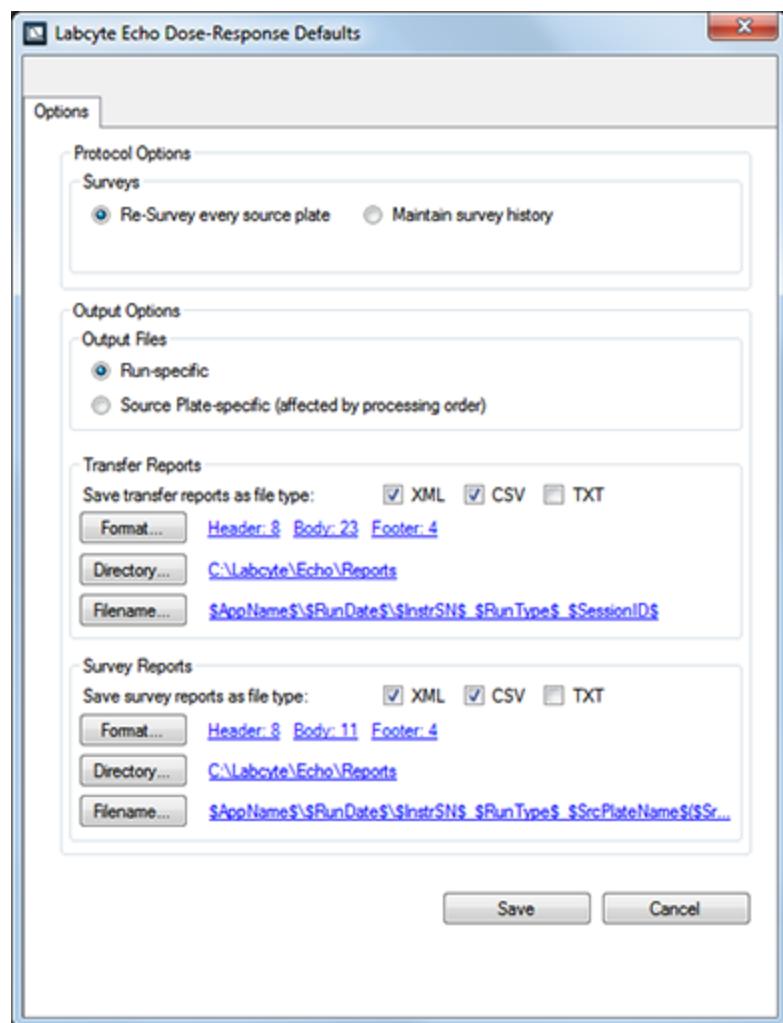
Preferences

The **Preferences/Options** window is accessible from the **Tools** menu. This window is very similar to the **Protocol Options** tab, but with the following differences:

- Settings are automatically applied to all protocols created unless changes were made in the **Protocol Options** window (report formats shown as “default”).
- Accessible from the **Toolbar**.

When the user clicks Preferences, the Options window opens to display the following protocol and output options:

Figure 64: Preferences/Options Window



Labware Definitions

The Echo Dose-Response application relies on the Labware definitions resident in the Echo Liquid Handler database. New plate definitions can be added or existing plate definitions can be managed by selecting **Labware Definitions** from the **Tools** menu.

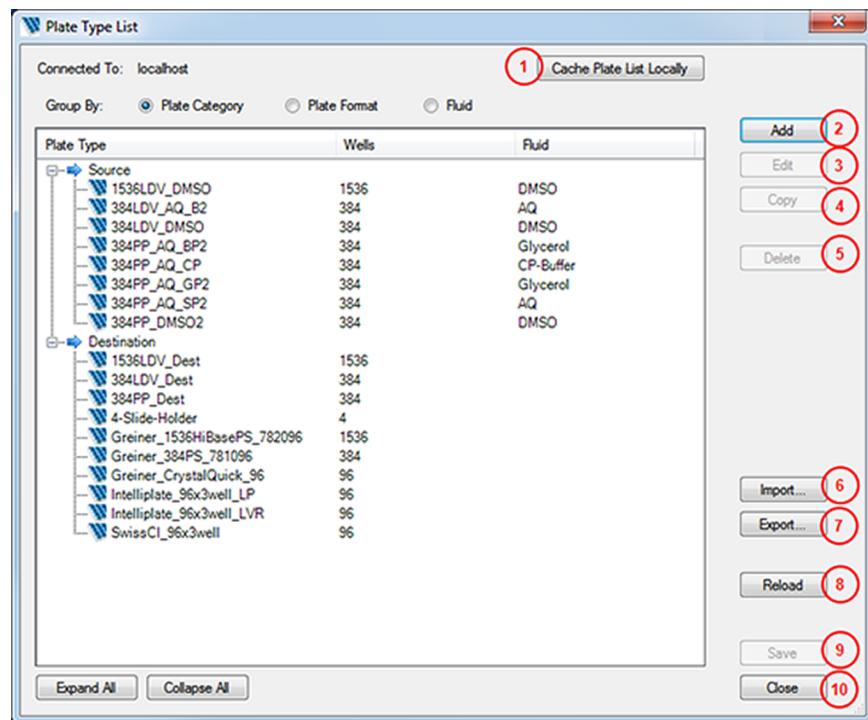
Each of the Labware topics are explained in the following topics.

- [Plate Type List](#)
- [Plate Type Editor](#)
- [Plate Export](#)

Plate Type List

The **Plate Type List** dialog box enables the user to add new plate definitions or manage existing ones.

Figure 65: Plate Type List dialog box



 **Note:** In case of an error message followed by a blank Plate Type List, connect to an Echo Liquid Handler or import the Labware file from the Echo Liquid Handler directory.

The table below describes the buttons/fields or sections in the **Plate Type List** dialog box and their functionality.

Table 5: Callout table for Plate Type List

Callout Number	Name	Description
1	Cache Plate List Locally	Click this button to save the plate list from the Echo Liquid Handler to the Echo® Dose-Response Application.
2	Add	Adds a plate definition. Only a destination plate can be added. Refer to one of the following options: <ul style="list-style-type: none"> • Add a Labware definition from the Echo® Dose-Response Application. • Add a plate definition from the Echo Liquid Handler software and reload the updated Labware definition to the Echo® Dose-Response Application.
3	Edit	Edit a plate definition. Select a plate definition and click the Edit button. <ul style="list-style-type: none"> • Source plate definition: Only the barcode location of the source plate can be edited. • Destination plate definition: Edit any setting in the definition
4	Copy	Copy a plate definition. Select a plate definition and click the Copy button. The user can copy a source or destination plate definition to create a destination plate definition. Alternate source or destination plate names can be created by copying existing plate types.
5	Delete	Delete a plate definition. Select a plate definition and click the Delete button. The user can delete a destination plate definition but not a source plate definition.
6	Import	Import a single plate definition or group of definitions from a specific file location. The file must have an .elwx extension to be imported.
7	Export	Export a single plate definition or group of definitions.
8	Reload	Reload the plate types that have been edited or deleted since the plate type list was last saved.
9	Save	Save any changes made to the plate type list, such as adding a new plate definition.
10	Close	Close the Plate Type List dialog box.

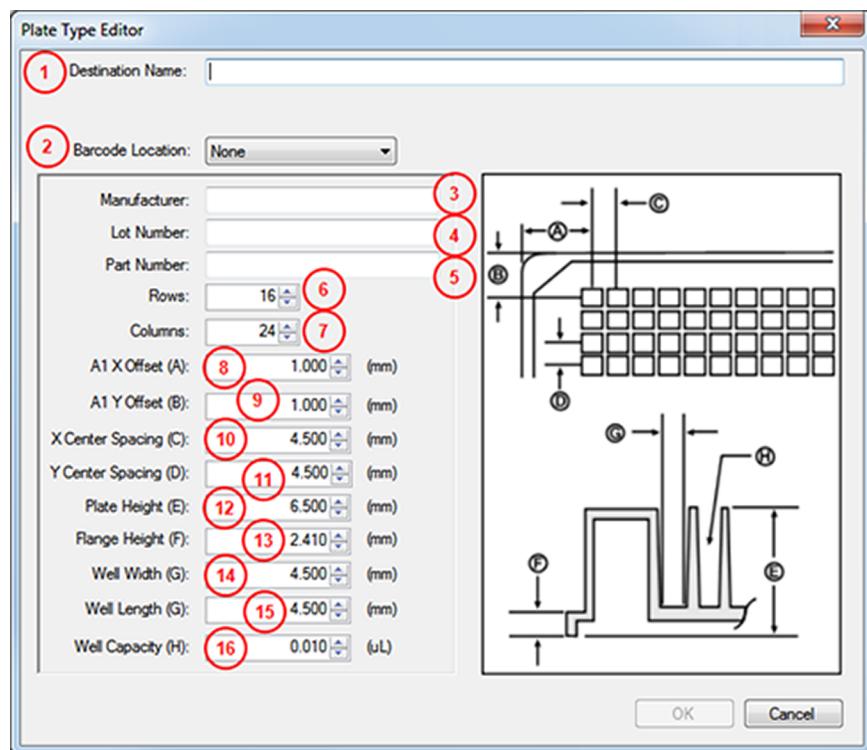


Note: In case of an error message followed by a blank Plate Type List, connect to an Echo Liquid Handler or import the Labware file from the Echo Liquid Handler directory.

Plate Type Editor

The **Plate Type Editor** dialog box is similar to the **Plate Specification** window in the Echo Liquid Handler software.

Figure 66: Plate Type Editor dialog box



Source plate definitions — Source plate definitions require exact specifications to accurately transfer nanoliter volumes; therefore, they are defined specifically for the Echo Liquid Handlers and tested at Labcyte (Echo-qualified). For this reason, source plates cannot be defined by the user. For existing source plates, only the barcode location can be edited. Contact Labcyte to add more source plates.

Destination plate definitions — The Echo Liquid Handler is programmed with several compatible destination plates; however, the user can add destination plates through the Echo® Dose-Response Application or the Echo Liquid Handler software.

The table below describes the buttons/fields or sections in the **Plate Type Editor** dialog box and their functionality.

Table 6: Callout table for Plate Type Editor

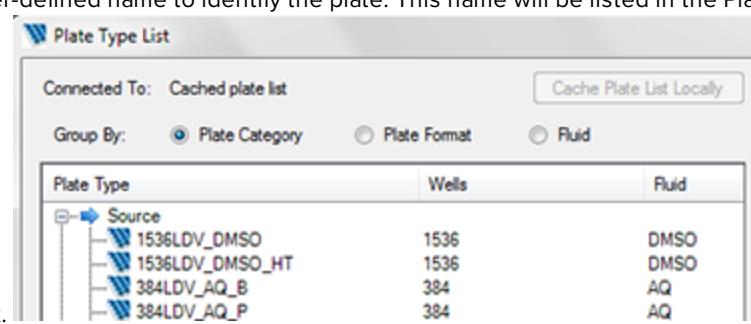
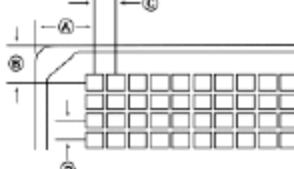
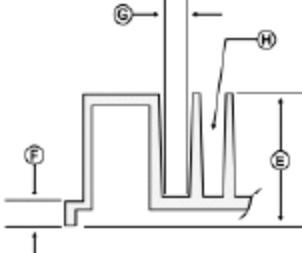
Callout Number	Name	Description
1	Name	User-defined name to identify the plate. This name will be listed in the Plate Type List dialog box. 
2	Barcode Location	Location of barcode label on the destination plate: <ul style="list-style-type: none">• None• Left

Table 6: Callout table for Plate Type Editor (continued)

Callout Number	Name	Description
		<ul style="list-style-type: none"> • Right • Long
3	Manufacturer	Name of the plate manufacturer.
4	Lot Number	Lot number assigned by the plate manufacturer.
5	Part Number	Part number assigned by the plate manufacturer.
6	Rows	Number of rows in the microplate.
7	Columns	Number of columns in the microplate.
8	A1 X Offset	(A)* Distance from left outside edge to center of first column where the left edge of the part will be defined as the two 12.7 mm areas (as measured from the corners) as specified in ANSI SLAS 1-2004 (R2012). Valid range: 0.0 to 128.0 mm.
9	A1 Y Offset	(B) Distance from top outside edge to center of first row where the top edge of the part will be defined as the two 12.7 mm areas (as measured from the corners) as specified in ANSI SLAS 1-2004 (R2012). Valid range: 0.0 to 86.0 mm.
10	X Center Spacing (C)	Column spacing: Each following row/column shall be an additional X.Y mm in distance from the top/left outside edge of the plate as specified in ANSI SLAS 4-2004 (R2012). Valid range: 0.05 to 9.0 mm.
11	Y Center Spacing (D)	Row spacing: Each following row/column shall be an additional X.Y mm in distance from the top/left outside edge of the plate as specified in ANSI SLAS 4-2004 (R2012). Valid range: 0.05 to 9.0 mm. 
12	Plate Height (E)	The overall height of the plate as specified in ANSI SLAS 2-2004 (R2012). Valid range: 6.5 to 14.5 mm.
13	Flange Height (F)**	The height of the flange (skirt) as specified in ANSI SLAS 3-2004 (R2012). Valid choices: 2.41 mm, 6.10 mm, and 7.62 mm.
14	Well Width (G)	The width of the well opening at the bottom (not an SLAS specified dimension). Valid range: 0.0 to 86.0 mm.
15	Well Length (G)	The length of the well opening (not an SLAS specified dimension). Valid range: 0.0 to 128.0 mm.
16	Well Capacity (H)	The overall capacity of the well in microliters. Valid range: greater than 0.0 uL. 



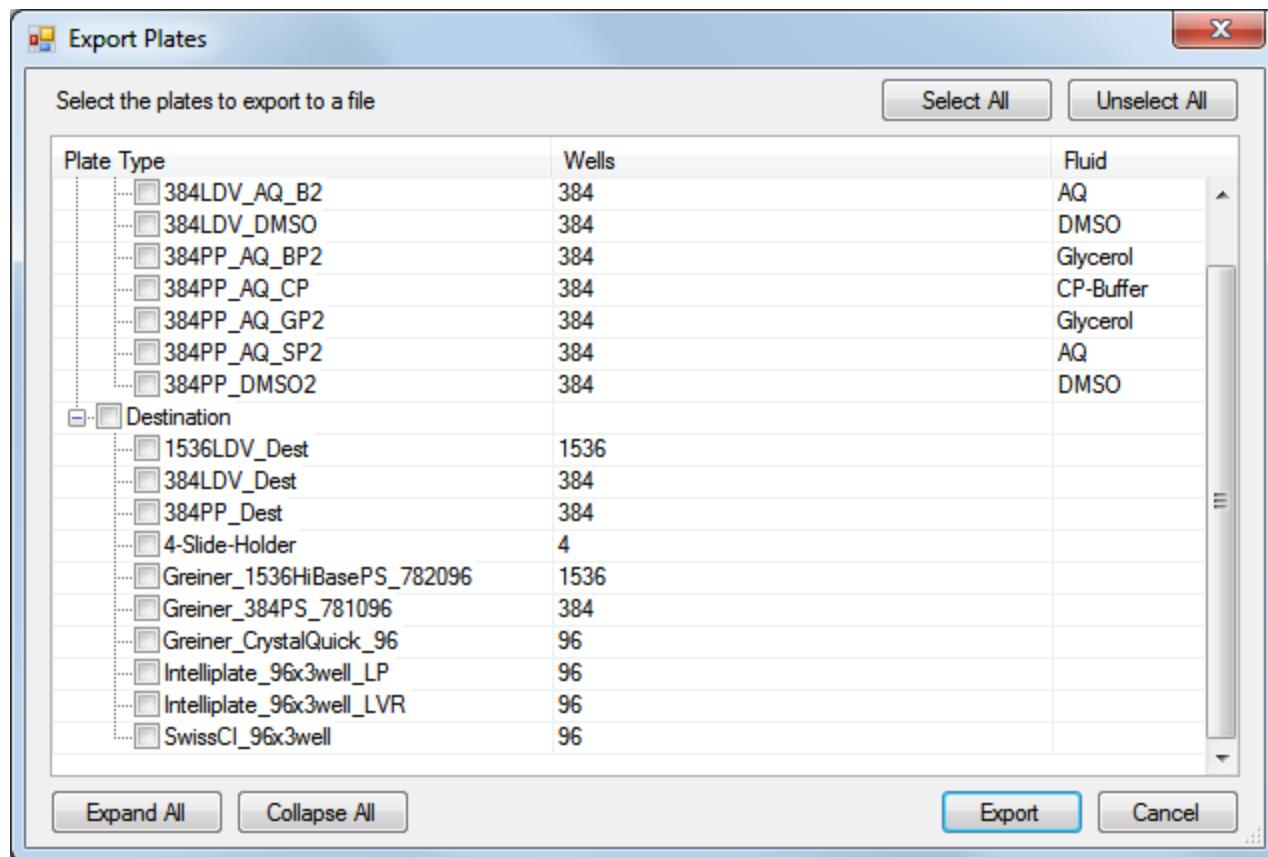
Note: * The items marked A-G are measurements that need to be taken by hand or filled in from the manufacturer's specifications. These dimensions and further details on their definitions can be found at <https://www.slas.org/resources/information/industry-standards/>.

** Currently, the Echo Liquid Handler does not use dimensions F (flange height) and H (well capacity). Any value entered for flange height or well capacity is ignored.

Plate Export

The **Export Plates** dialog box displays the plates currently available in the Echo® Dose-Response Application. The user can select and export one or more plates to a file (.elwx file extension) for use in another application.

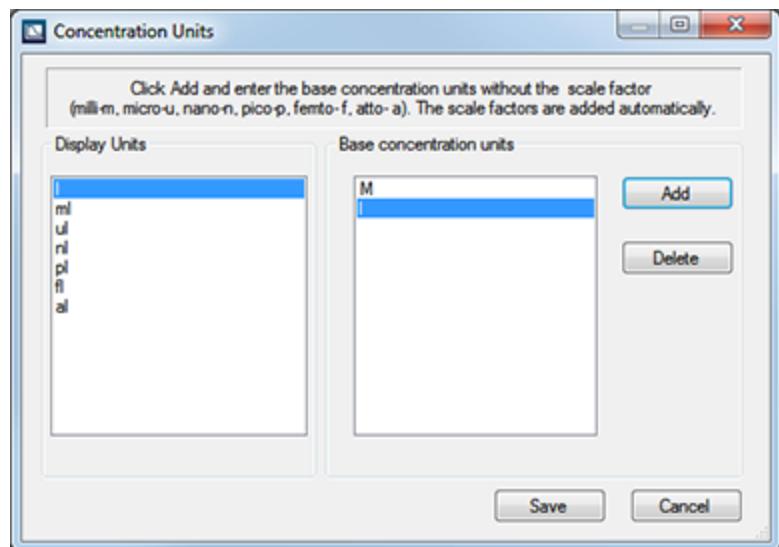
Figure 67: Export Plates dialog box



Concentration Unit Definitions

The **Concentration Unit Definitions** window is accessible from the **Tools** menu. This window is used to create custom concentration unit definitions.

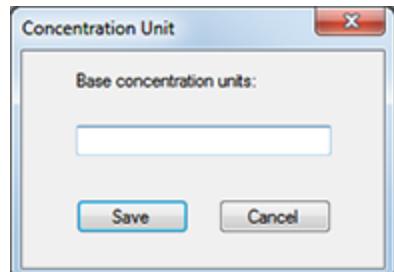
Figure 68: Concentration Units dialog box



To add custom concentration units:

1. Click **Add** in the **Concentration Units** dialog box.
2. Type the letter(s) for the new concentration unit and click **Save**.

Figure 69: Concentration Unit dialog box

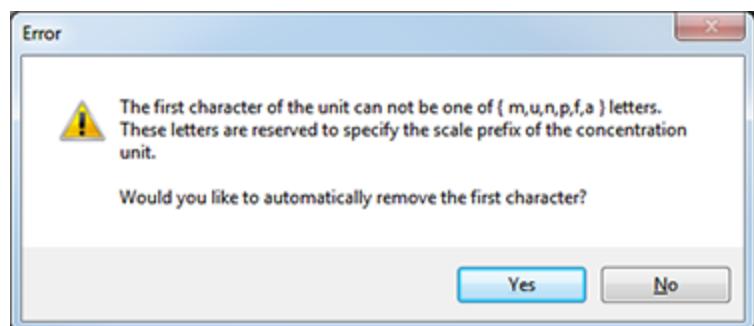


3. Click **Save** to save the new concentration unit and exit.



Note: If a reserved letter was entered for the new concentration unit, the error box below is shown and the concentration unit is not added.

Figure 70: Concentration Unit Error dialog box



To delete custom concentration units:

1. Select the custom concentration unit to be deleted in the **Base concentration units** list and click **Delete**.
2. Click **Yes** in the **Confirm Delete** dialog box.
3. Click **Save** to save the changes and exit.

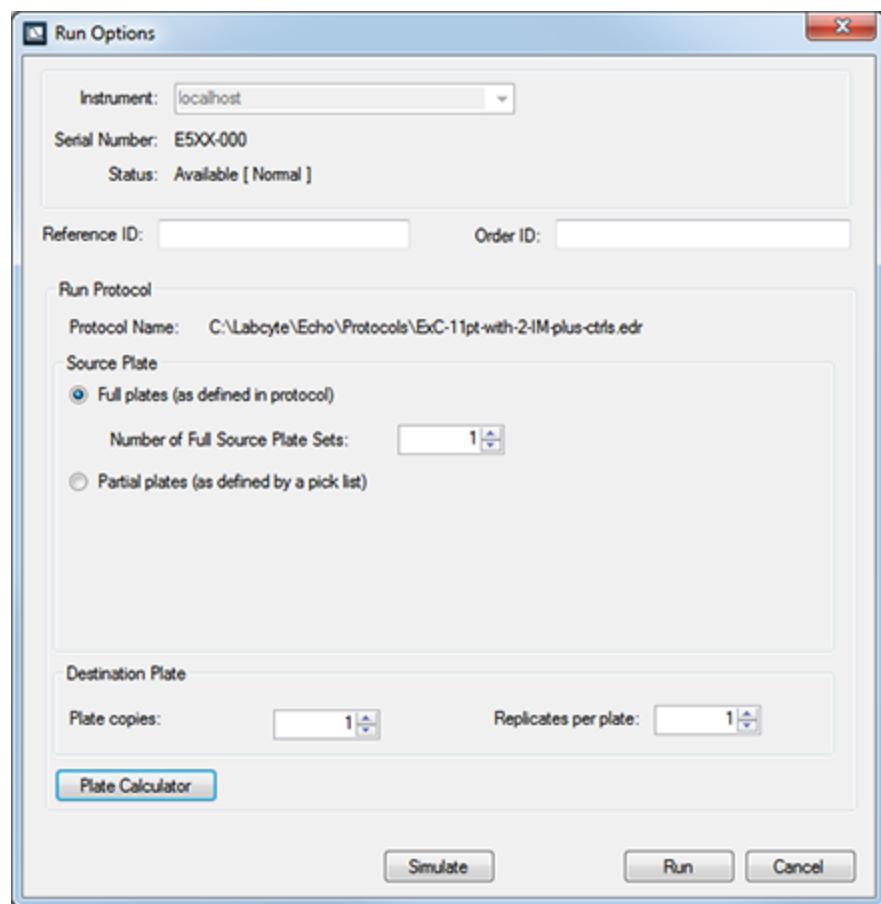


Note: If the user clicks **Cancel** instead of **Save** in the **Concentration Units** dialog box, the custom concentration unit is not deleted.

Run Protocol

The **Run Options** dialog box enables the user to run a simulation of the transfer protocol or run the transfer protocol on the Echo instrument. If the application is not connected to an Echo instrument, this dialog box also provides a connection dialog box.

Figure 71: Run Options dialog box



To run a protocol:

1. Select **Run** from the **Protocol** menu or click the **Run** icon in the **Toolbar**.
2. Select the **Instrument** if it is not already selected.
3. Optionally, enter a **Reference ID** and/or **Order ID** to be used to reference the run in the output report files.
4. For the **Full Plates** option defined by the protocol, specify the number of source plates to process. The number of source plates are defined in the protocol and cannot be overridden in the **Run Options** dialog box.
5. Click **Partial Plates** to pick specific plates. When the **Partial Plates** option is selected, an **Import** button is displayed to import a .csv pick list indicating the wells to be transferred. For more information on how to define a pick list, see [Importing a Pick List](#).



Note: If the **Partial Plates** option is selected, the **Remove duplicate samples** and **Pre-process pick list in order specified** options become available.

- Select **Remove duplicate samples** to eliminate redundant samples found in the imported pick list. This may occur in cases where an output file has been imported as an input file. For example, when importing the Exceptions Report as a pick list to retry missed transfers.
- Select **Pre-process pick list in order specified** to automatically optimize the order of transfers to destination plates before the run starts. This is only recommended for use with plates that can be

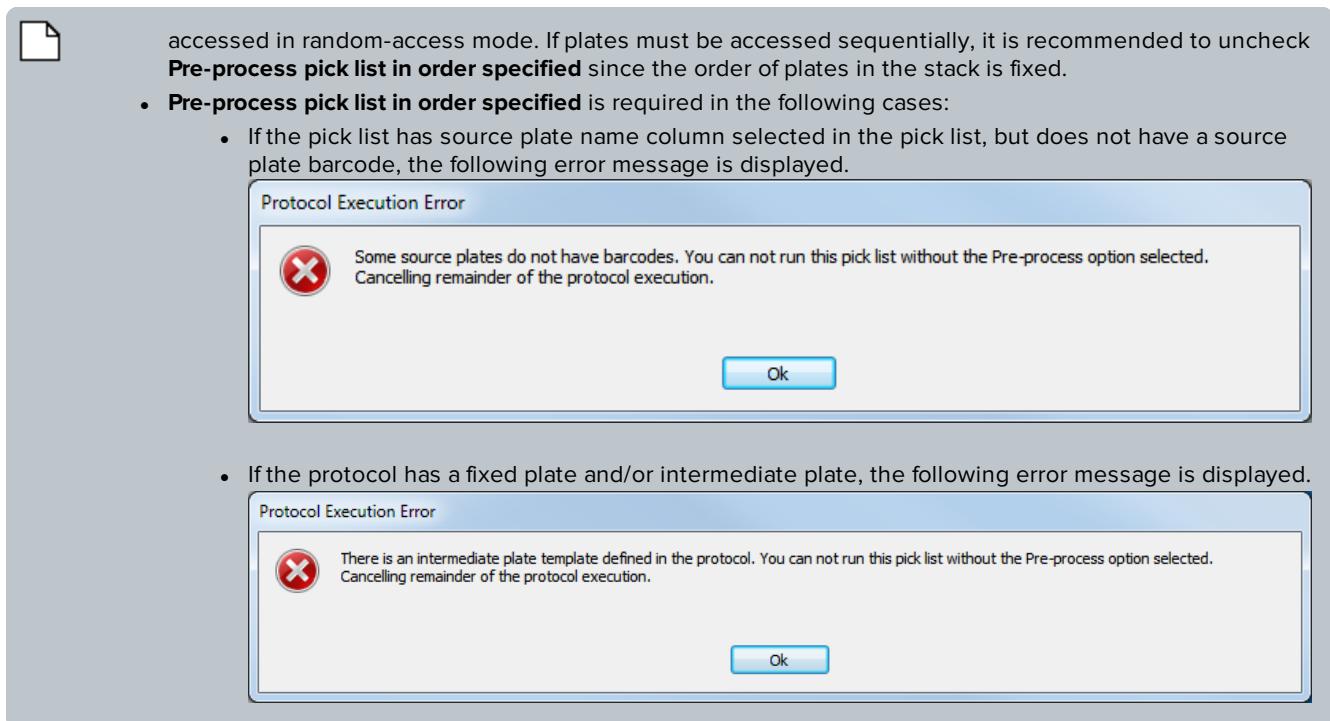
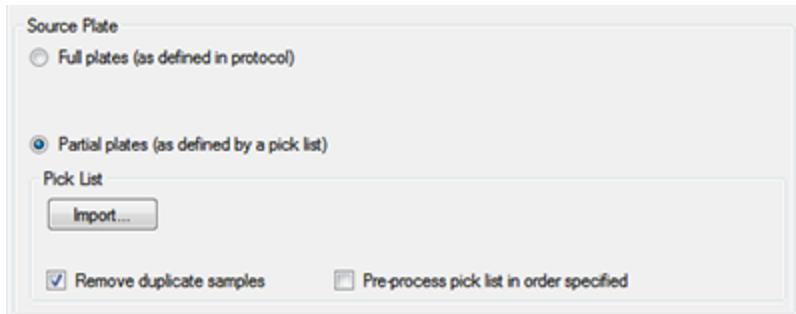


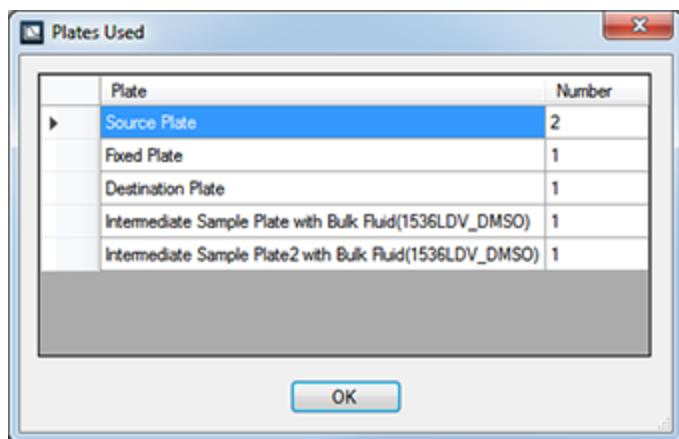
Figure 72: Run Options dialog box



7. In the **Destination Plate** section, change the value for **Plate copies** to override the number of plate copies for the protocol. Change the **Replicates per plate** value to override the original setting in the protocol for the replicates per plate.

8. Click the **Plate Calculator** button to view the number of plates needed to run the protocol.

Figure 73: Plates Used dialog box



9. Optionally, click **Simulate** to test the transfer protocol.



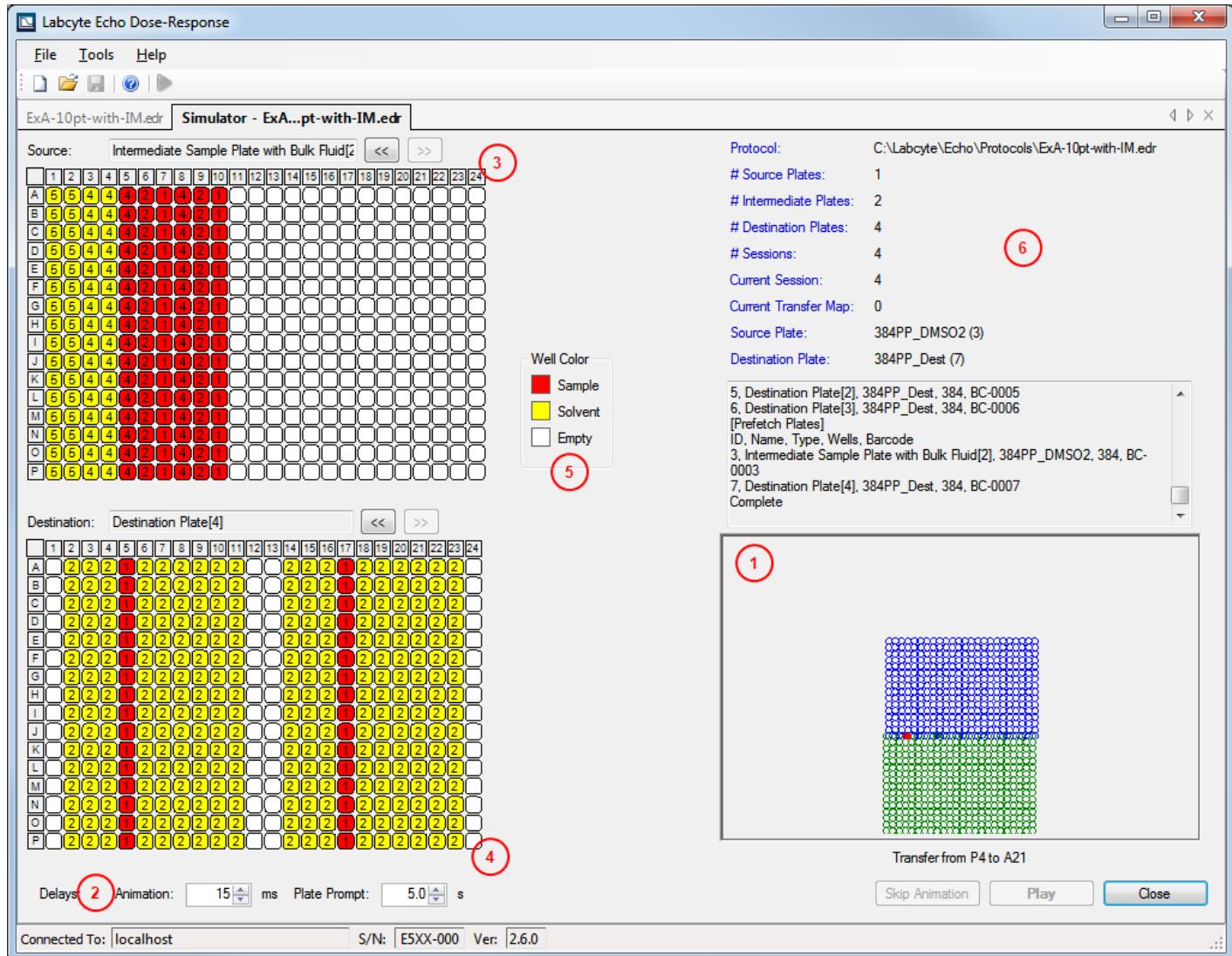
Note: Running a simulation is recommended to verify the transfers defined in the protocol before a live run.

10. Click **Run** to execute the protocol.

Simulator Window

The simulator is an animation of fluid transfer for the selected protocol. The simulation is interactive and allows the user to respond to a prompt box to insert and remove plates. Since this is a simulation, the prompt box appears for only 5 seconds, then the software automatically responds to the prompt and continues to the next step.

Figure 74: Simulator Window



The simulation is very useful for testing a newly created protocol and should be run before committing time and material to the actual transfer run. Since the simulation runs very quickly, several different plate configurations can be tested in a short time.

The simulation can also be used to assemble the plates needed for the transfer protocol. The protocol data in the upper right area of the screen displays the number of source, intermediate (if used), and destination plates that are needed to run the protocol.

The table below describes the buttons/fields or sections in the **Simulator Window** and their functionality.

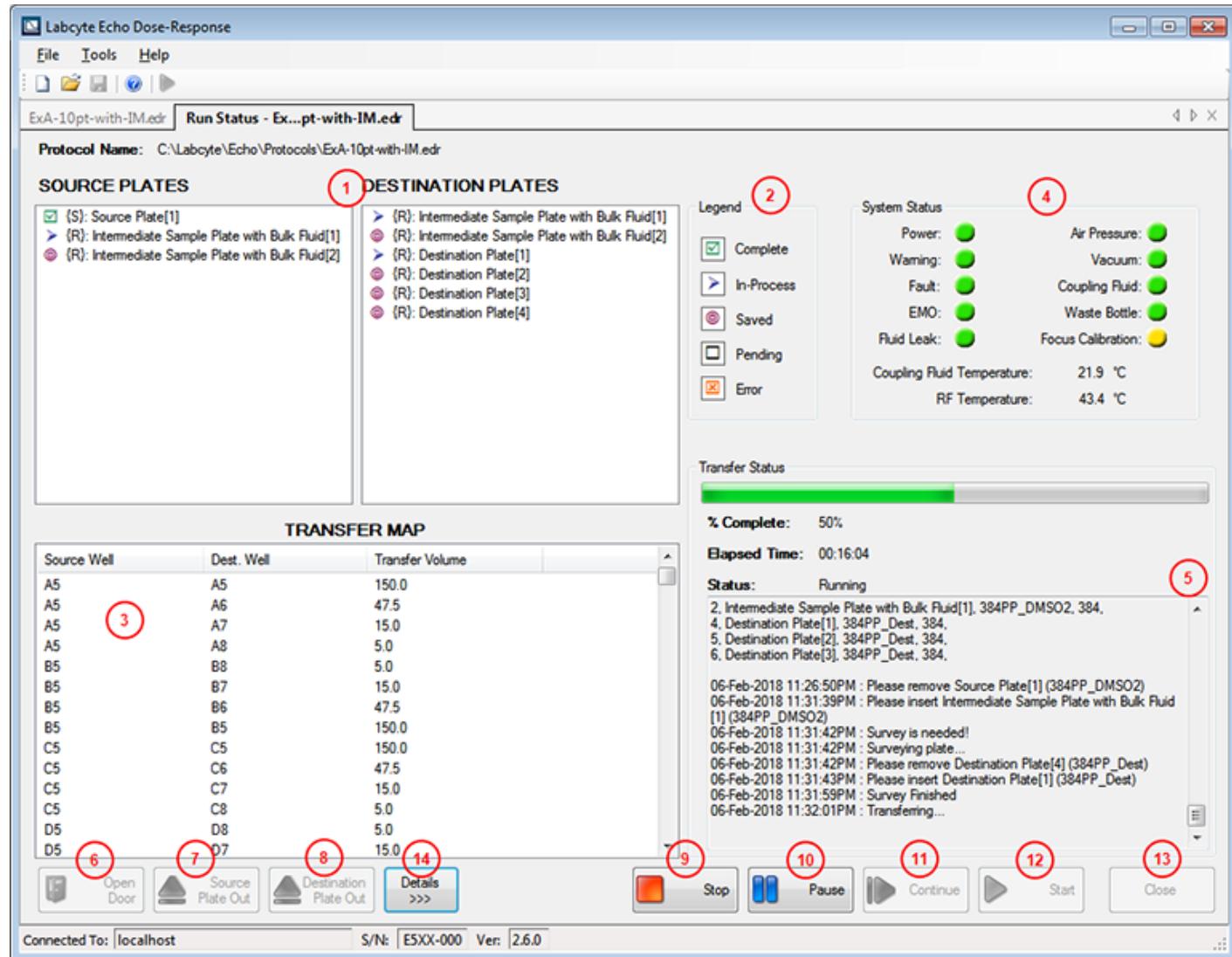
Table 7: Callout table for Simulator Window

Callout Number	Name	Description
1	Transfer Animation Box	Simulates the movement of the destination plate (green) as it receives liquid from the source plate. Each source-to destination well transfer is documented below the plates.
2	Delays	<p>The following Delays commands are available to manage the transfer animation.</p> <ul style="list-style-type: none"> • Animation (milliseconds) — Controls the speed of the simulated well-by-well transfer. Speed ranges from 500 ms to 0; default is 15 ms. Higher speeds are useful to slow down the animation when transfers jump around the plate. For example, during backfills. • Plate Prompt (seconds) — Controls the time interval that the plate prompt box is displayed, from 30 s to 0. Zero setting is useful to bypass the prompt box entirely and view the animation only. • Skip Animation — Bypasses the animation step and shows only the transfer results for that step. This control enables the user to quickly review the plate mapping after each transfer step. <p> Note: This control does not bypass plate prompts or bulk fill displays.</p> <ul style="list-style-type: none"> • Play — Begins the simulated fluid transfer. • Close — Closes the simulation window. If a simulation is in progress, it will need to be canceled before the window can be closed.
3	Source Plate Type Map Box	Shows the source plate that is selected for transfer. As the simulation runs, each source well shows the number of times a transfer is made from that well.
4	Destination Plate Type Map Box	Shows each well that receives transfers and the number of transfers that are received.
5	Well Color Legend	Indicates the colors used to represent the contents of the wells in the source, intermediate, and destination plates. <ul style="list-style-type: none"> • Red — Sample • Yellow — Solvent • Blue — Control • White — Empty
6	Protocol Information Box	Provides details about the transfer protocol, such as the number of plates that will be needed. As the simulation proceeds, this box is updated to show which plates are currently in use.

Run Status Window

The **Run Status** window is a dynamic window that provides control buttons to run the protocol, and various status windows to show the progress of the compound transfer, the state of the Echo instrument, and the final well positions and volume of the transferred fluid.

Figure 75: Run status window



The table below describes the buttons/fields or sections in the **Run Status** window and their functionality.

Table 8: Callout table for Run Status window

Callout Number	Name	Description
1	Source and Destination Plate windows	Shows the status of each plate as it progresses through the transfer protocol. The Legend interprets the symbols used in the status windows.
2	Legend	Identifies each of the states (Complete, InProcess, Saved, Pending, Error) possible for a source and destination plate during a run.
3	Transfer Map window	Shows the details of each well-to-well transfer.
4	System Status window	Shows the status of the Echo instrument during the transfer run. If a problem occurs, an alert is indicated by the colors turning from green to yellow to red. If it is red, the user needs to do something to fix it.
5	Transfer Status window	Shows a progress bar of the transfer protocol, as well as percent completion and elapsed time. This section also provides a text window that displays all the actions that occur during the run.
6	Open Door button	Opens the process door of the connected Echo Liquid Handler.
7	Source Plate button	Extends the source plate gripper.
8	Destination Plate Out button	Extend the destination plate gripper.
9	Stop button	Stops the transfer protocol that is in progress.
10	Pause button	Pauses the transfer protocol that is in progress.
11	Continue button	Continues the transfer protocol that is in progress.
12	Start button	Begins the transfer protocol.
13	Close button	Closes the Run Status window.
14	Details button	Shows the debugging information for the protocol run.

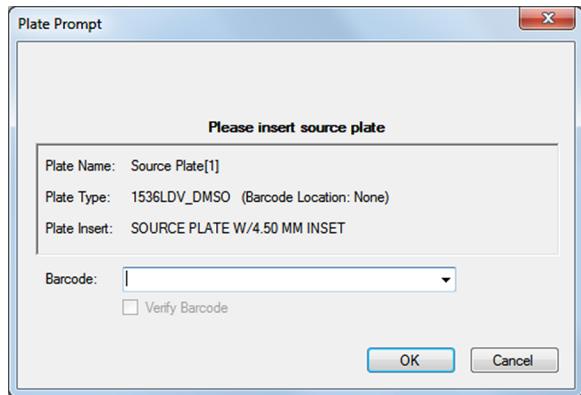
Plate Prompt

The **Plate Prompt** boxes instruct the user to insert or remove a plate. Depending on the protocol, there may be multiple source and destination plate prompts displayed and the order in which they are shown is also dependent on the protocol.

The plate prompt steps are described below:

1. Insert the source plate into the source plate gripper stage and click **OK**.

Figure 76: Insert source plate prompt box



Identify the source plate in one of the following ways:

- **Plate ID** — Select the **Source Plate ID** from the drop-down menu.
- **Barcode** — Select the **Barcode** field and ensure the **Verify Barcode** option is selected. The Echo instrument automatically scans the barcode label on the plate and inserts it into the **Barcode** field.



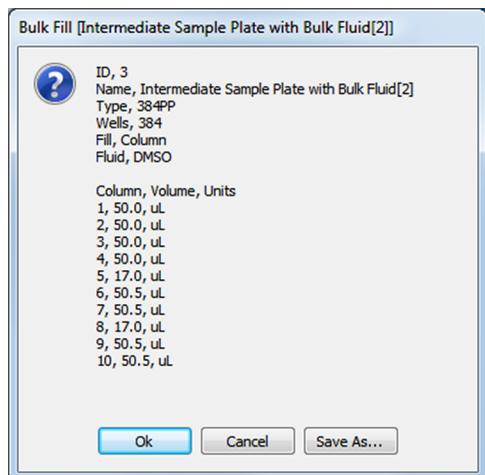
Note: If the **Barcode** field was skipped and **Verify Barcode** was not selected, the software displays a plate read error.

- **Pre-processed pick lists** — Ensure that the pre-processed pick lists option is selected in the Run Option window. If this option was selected, the Barcode field is initialized to display source plates listed in the pick list; therefore this field is not available for text changes (grayed out). However, the **Verify Barcode** option can be disabled to bypass the barcode scan — particularly if user-created plate IDs are being used instead of barcodes to identify the source plates.

After the source plate is processed, the software removes it from the drop-down menu.

2. Before the intermediate plate is inserted, the **Bulk Fill** dialog box is displayed if **Offline Bulkfilling** was selected. Click **OK**.

Figure 77: Bulk Fill dialog box

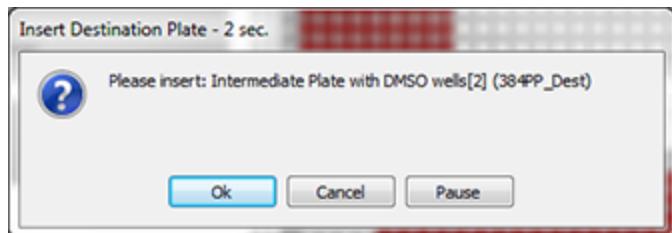




Note: The information presented in the **Bulk Fill** dialog box can be saved to a file and used to pipet solvent into the bulk fill wells of the intermediate plate.

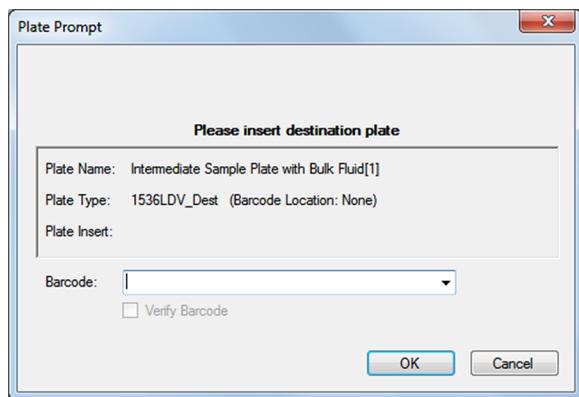
3. Insert intermediate plate and click **OK**.

Figure 78: Intermediate Plate dialog box



4. Insert destination plate and click **OK**.

Figure 79: Insert destination plate prompt box

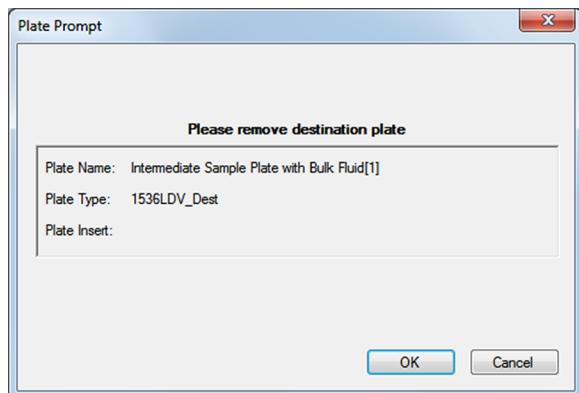


- Select the **Source Plate Barcode** (or plate ID) from the drop-down menu. If there is only one destination plate, the software automatically selects it and disables the **Verify Barcode** option.
- Select **Verify Barcode** (optional) and click **OK**.

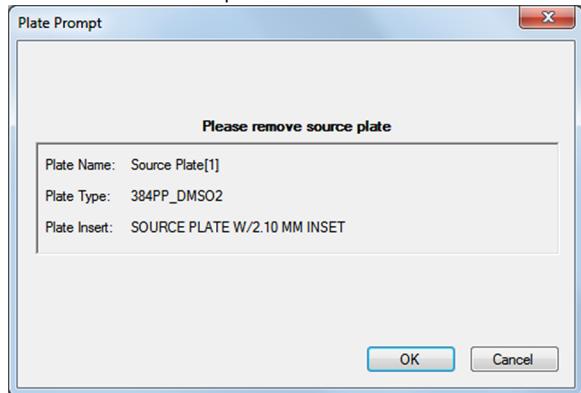
After the destination plate is processed, the software removes it from the drop-down menu.

5. Remove the destination plate and click **OK**.

Figure 80: Remove destination plate prompt box



6. Remove the source plate and click **OK**. Remove source plate prompt box



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