Muse™ Cell Analyzer

User's Guide

For Research Use Only; not for use in diagnostic procedures.

0110-7895 Rev C

Disclaimer

EMD Millipore reserves the right to change its products and services at any time to incorporate the latest technological developments. This guide is subject to change without notice.

EMD Millipore has not validated the analysis of Muse™ System data using third-party programs and cannot warrant that the results using these programs will be correct. EMD Millipore does not provide support for any third-party programs.

Limitations

The Muse™ System is for research use only; not for use in diagnostic or therapeutic procedures.

The results of the assays are dependent upon the proper use of the reagents and instrument. Please refer to the appropriate reagent kit user's guide for specific instructions and limitations.

Trademarks

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Patents

U.S. patents 5,798,222; 6,816,257; 6,710,871 and foreign equivalents owned by EMD Millipore Corporation.

Patent App No. 61482946 owned by EMD Millipore Corporation.

The Muse™ Count & Viability Reagent is the subject of U.S. Patent 6,403,378 owned by EMD Millipore Corporation.

Contents

Preface

The Muse™ System is the first compact, easy-to-use, desktop cell analysis system that can perform a wide range of cellular assays.

Assay and Reagent Overview

For information on performing the assay protocols, refer to the specific kit user's guide, which can be found at www.millipore.com/muse. For order information, see page 101.

For Research Use Only; not for use in diagnostic procedures.

- **Muse System Check Kit**

  Verifies the performance of the system by assessing counting accuracy and fluorescence detection using a standardized fluorescent bead reagent. The kit contains a bead reagent and diluent.

- **Muse™ Count & Viability Kit**

  Used to determine viability and total cell count. Accurate assessments can be made with a wide variety of cell lines, even those with unusual culture conditions or a tendency to aggregate.

- **Muse™ Count & Viability Reagent (200X)**

  Enables accurate counting and discrimination of viable and nonviable cells for a variety of difficult cell sample.

- **Muse™ Annexin V & Dead Cell Kit**

  Used to assess early and late apoptosis. The assay relies on the translocation of phosphatidyl serine (PS) to the outer surface of the cell membrane, an event often associated with the onset of apoptosis.

- **Muse™ Cell Cycle Kit**

  Identifies and measures the number of cells within the various phases (G0/G1, S, and G2/M) of the cell cycle.

- **Muse Cell Dispersal Reagent**

  Enzymatic reagent that gently disaggregates clumped cells in suspension, improving the accuracy and precision of cell counts. The formulation of CDR has been optimized for use with Muse™ Count & Viability Reagent.

About this Guide

The *Muse™ Cell Analyzer User's Guide* provides detailed information on operating and maintaining the Muse™ System. This guide provides instructions for setting up the system, managing users and data files, running a System Check procedure, and cleaning the system. It does not include instructions for using the software modules to acquire samples and analyze data.

For information on preparing samples and running them on the instrument, refer to the specific kit user's guide located at www.millipore.com/muse.

Conventions Used in This Guide

■ **NOTE:** Points out additional information that may be helpful.

▲ **WARNING:** Alerts you to situations that could result in bodily harm, instrument damage, failure in a procedure, or incorrect results.

Help

1 Read through the section of the guide specific to the operation you are performing. Refer to the table of contents and index to locate information. A glossary is included to assist you with any unfamiliar terms.

2 See the troubleshooting section for a list of problems and suggested solutions.

3 Refer to the technical support contact information below:

- For ordering information or technical support, call toll-free in the USA and Canada:
  Phone: +1 (800) MILLIPORE (645-5476)
  Fax: +1 (800) 645-4539
- Outside the US, visit www.millipore.com/offices for up-to-date worldwide contact information.

4 For information on performing an assay, refer to the specific kit user's guide, which can be found at www.millipore.com/muse.

Safety

The Muse™ System is equipped with safety features for your protection. Use the system only as directed in this user's guide. Do not perform instrument maintenance or service except as specifically stated. Read the following safety information before using the system.

General Safety

▲ **WARNING:** If this instrument is not used in the manner indicated by the instructions in this guide, the safety features of the instrument may be impaired. Follow these guidelines:

- Use only the tubes specified. The use of tubes other than those specified may result in damage to the instrument.

Biological Safety

▲ **WARNING:** All biological specimens and materials that come into contact with them can transmit potentially fatal disease. To prevent exposure to biohazardous agents, follow these guidelines:

- Handle all biological specimens and materials as if capable of transmitting infection. Dispose of waste using proper precautions and in accordance with local regulation. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.
- After emptying waste bottle, add bleach to the first (fill) line. It takes approximately 10 mL of bleach to reach the fill line. Dispose of waste in accordance with federal, state, and local regulations.

Electrical Safety

▲ **WARNING:** Power off the instrument using the Power Options tool on the main menu and disconnect the power cord before replacing fuses.

Laser Safety

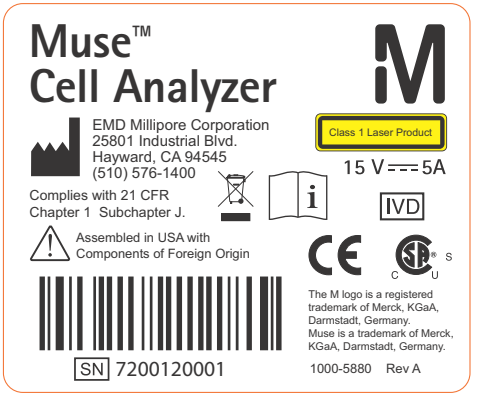
The Muse™ System contains a Class IIIb laser operating at 532 nm in CW mode. Light shields within the instrument enclose the path of laser radiation. Additionally, the instrument enclosure provides secondary protection from any laser radiation.

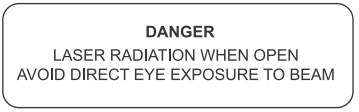
▲ **WARNING:** To avoid exposure to laser radiation or electric shock, follow these guidelines:

- Do not open the instrument or attempt to perform any internal maintenance. There are no user serviceable parts.
- Turn off the power to the system before removing the flow cell.

Precaution Labels

The following labels are affixed to the Muse™ System.





Limitations

- The Muse™ System is for research use only; not for use in diagnostic or therapeutic procedures.
- The results of the assays are dependent upon the proper use of reagents and instrument. Refer to the appropriate kit user's guide located at www.millipore.com/muse for specific instructions and limitations.

Introduction

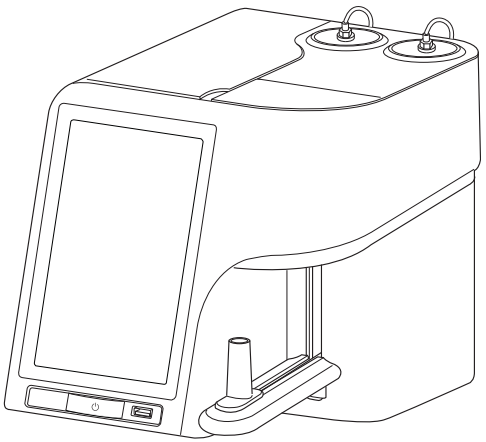
Muse™ System

The Muse™ System consists of a compact, portable, and easy-to-use cell analyzer, software, and optimized reagents. The Muse™ Cell Analyzer uses patent-pending, miniaturized fluorescent detection and micro-capillary technology to deliver quantitative cell analysis of both suspension and adherent cells 2 to 60 µm in diameter. The software includes a dedicated module for each assay, as well as a tools for verifying instrument performance and cleaning the instrument's fluid system.

Convenient mix-and-read assays are optimized for accuracy and convenience. The available assays include:

- Count & Viability
- Annexin V & Dead Cell
- Cell Cycle

Refer to the assay user's guide for detailed instructions on preparing samples and running an assay using the assay-specific software module.



Muse™ Cell Analyzer

Setting Up Your System

Your Muse™ System is easy to install. Simply plug it in and start operating.

Components

Your Muse™ System shipped with the following components:

- Muse™ Cell Analyzer and power cord
- cleaning and waste bottles and corresponding color-coded tubing
- flow cell
- USB flash drive with software recovery files and user's guide

Unpacking the Instrument

Follow the instructions in the Quick Start Guide to unpack and set up your instrument. Here are the steps outlined briefly.

1. Remove the instrument from the shipping box. You may wish to save the box and foam insert in the event the instrument needs to be moved to a different location.
2. Place the unit on a stable surface. Although it is compact and portable, it contains precisely aligned optical components that are sensitive to jarring movements.
3. Connect the color-coded fluid tubing to the back of the instrument.
4. Add 10 mL of bleach to the waste bottle and fill the cleaning solution bottle to the indicator line with Guava ICF. Place the fluid bottles in their respective receptacles. Match the icon on the fluid bottle with the icon in the receptacle.
5. Connect the fluid tubing from the back of the instrument to the top of the corresponding bottle.
6. Insert the flow cell. See "Replacing the Flow Cell" on page 73 from step step 4 on for details.
7. Plug the instrument into a grounded three-prong AC outlet.

   When you plug in the instrument for the first time, it will automatically turn on.
8. Log on as an Administrator. See "Logging On as the First User" on page 35.

9 Perform a Complete System Clean to prime the fluid system. See "Performing a Complete System Clean" on page 61.

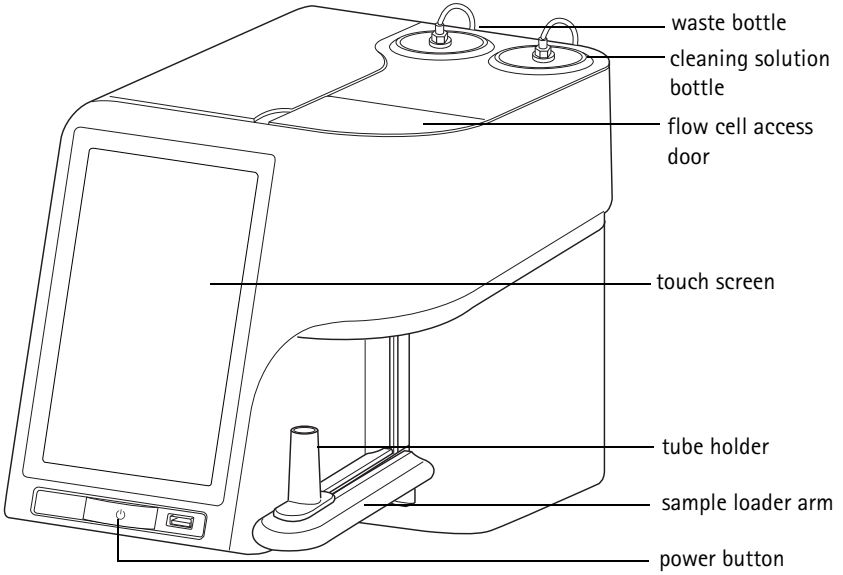
10 Perform a System Check procedure. See "Running a System Check Procedure" on page 43.

System Overview

Muse™ Instrument Overview

The Muse™ Cell Analyzer is a compact instrument for fluorescence-based, three parameter cell analysis. It is designed for easy and intuitive operation and minimal maintenance. The integrated touch screen provides an easy-to-use software interface for operating the system.

To turn on the instrument, press the power button located below the touch screen on the front of the unit. To turn off the instrument, use the Power Options feature on the main menu displayed on the touch screen. Five USB ports—one below the touch screen and four on the back of the unit—allow you to connect a Muse™-compatible printer, USB flash drive, and/or keyboard and mouse. For a list of approved printers, see "Approved Printers" on page 104.



Sample Loader

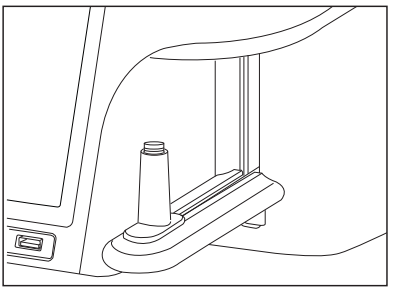
The sample loader holds an individual sample tube. The loader arm can be lifted easily with your finger and released with the touch of a button using the touch screen. The following tube is supported:

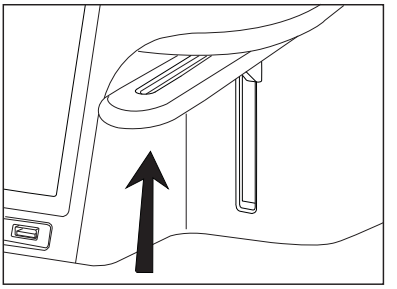
- 1.5-mL microcentrifuge tube with conical tip and screw cap (if snap-cap tubes are used, cut off the cap)

The system automatically detects tubes with caps and will not load the tube if you install a tube with a cap.

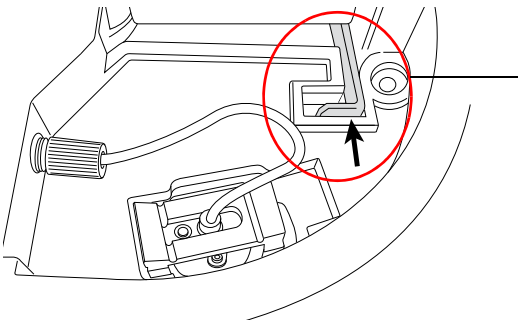
Loading and Unloading a Tube

To load a tube, place the tube in the tube holder and lift up on the loader arm. As you lift the arm, the tube automatically slides back to the loaded position and is seated under the capillary.





During a run, the loader arm drops automatically when sample acquisition is complete. To manually unload a tube, use the eject button  on the touch screen. Additionally, a lever located in the flow cell access door allows you to drop the loader arm to remove a sample tube in the rare event of a power outage. Open the flow cell access door and push back on the switch shown in the following illustration.

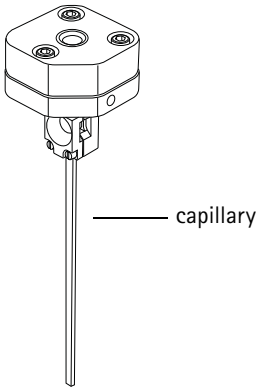


Cleaning Solution and Waste Bottles

The cleaning solution bottle can be filled with Guava Instrument Cleaning Fluid (ICF) for system cleaning. For information on filling the bottle, refer to "Filling the Cleaning Solution Bottle" on page 70. The waste bottle captures the sample fluid after it exits the fluid system. Add 10 mL of bleach to the waste bottle after you empty it and before installing it in the unit. For information on emptying the waste bottle, refer to "Emptying the Waste Bottle" on page 71.

Fluid System

Sample uptake occurs through the capillary part of the flow cell assembly. Sampling is regulated by a variable-speed fluid pump. The pump does not require sheath fluid or supplementary fluids for sample acquisition.



Because the system's sampling precision depends on the integrity of the fluid pathway, it is important to maintain a clean system. Do not allow samples to remain in the capillary for extended periods of time as they may eventually clog the system. Perform frequent cleaning cycles to prevent the build-up of cellular debris that may restrict sample flow. If a clog does occur, you can clear it by using the backflush feature, which reverses the flow of fluid and flushes it out of the capillary at high speed. See "Backflushing the Capillary" on page 60 for detailed instructions on using the backflush feature.

At the start of each day perform a Complete System Clean to prime the fluid system. See "Performing a Complete System Clean" on page 61.

Always leave a tube of deionized water on the instrument when not in use.

Software Overview

Use the software to acquire samples, view results, and control the instrument. In addition, each software module allows you to adjust the markers and/or gates to fine-tune the analysis. Instructions for running samples using the individual assay modules can be found in the reagent kit user's guide for each individual assay kit.

Navigating Through the Software

A navigation bar at the top of the screen indicates where you are in a given procedure. The current step is highlighted gray. These buttons vary depending on the procedure you are performing (for example, an assay, Complete System Clean, or System Check procedure).

If you access one of these procedures and wish to exit, simply press **Home** to return to the main menu. If the screen you're on does not display a navigation bar, select the Back, Close, or Cancel button from the current screen to access a screen with a navigation bar.

For simplicity, the navigation bar has been cropped out of most of the screens appearing in this guide.

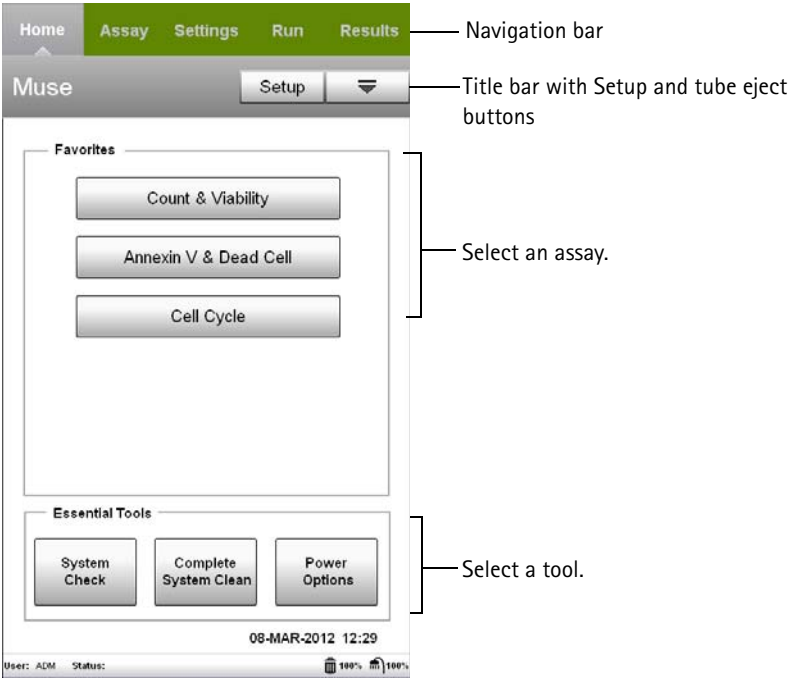
Navigation bar

A status bar at the bottom of the screen indicates the user currently logged on, instrument status, and the volume remaining in the cleaning bottle and capacity remaining in the waste bottle.

Example shows waste bottle has 23% capacity remaining (77% full) and cleaning bottle has 20% of the solution remaining (80% has been used).

Main Menu

The main menu allows you to choose an assay or an Essential Tool.



Below the navigation bar, is the title bar containing the Setup button and eject button  . The eject button releases the sample loader arm, allowing you to manually unload a tube. Setup, which is accessible from the main menu only, allows you to set specific system features such as managing users and data, and setting specific software options. See "Setup" on page 77 for details on the Setup menu.

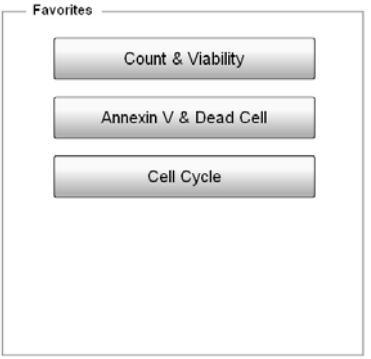
Title bar

The lower part of the screen is divided into two main sections—Favorites (assays) and Essential Tools. Favorites allows you to select an assay to run. Essential Tools allows you to check the system performance, clean the system, and power off or log off of the system.

Favorite Assay Buttons

The main menu allows you to select from the following assays:

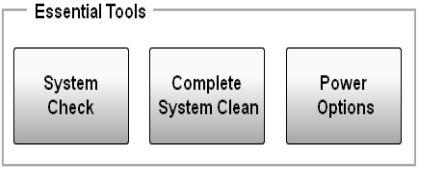
- **Count & Viability** for performing cell counting and viability assays
- **Annexin V & Dead Cell** for performing apoptosis assays
- **Cell Cycle** for performing cell cycle assays



Essential Tools

The following tools are available:

- **System Check** to check the system's counting and fluorescence performance prior to running samples. The System Check Beads are standard particles used with this tool. For more information on running System Check, see page 43.
- **Complete System Clean** to clean the fluid system. For information on this cleaning procedure, see "Performing a Complete System Clean" on page 61.
- **Power Options** to turn off the system or log off as the current user



Entering and Selecting Information

The touch screen provides various ways of entering and selecting information, most of which are intuitive. If you connect an external mouse, you can use the cursor to make selections.

- Touch buttons, such as the assay buttons on the main menu.
- Drag sliders to increase or decrease values, or drag handles to adjust gates and markers.
- Use the keypad to enter information into text or numeric fields. Touch the text field to automatically open the keypad. Type the information and select **Done** or touch outside the keypad to close it.

Files

For each assay, the software saves two files: an FCS 3.0 data file and a CSV spreadsheet results file, each containing data for all samples in the run. Additionally, you can optionally choose to save a separate file of the instrument and analysis settings.

FCS 3.0 Data Files

A single FCS file includes the results and sample information for all samples acquired within a run, as well as an event log

At the completion of the run (after you click **Finish** to save and close the data set), you can enter a new file name or use the default file name that appears. The default file name is the user's initials, followed by the date and time (for example, PT_15Feb2012_115618.FCS). The file name assigned to the FCS file is also assigned to the spreadsheet results file. An extension is automatically appended to the file name you enter. The first three characters represent the assay type, followed by FCS.

Assay	**File name**
Count & Viability	filename.VIA.FCS
Annexin V & Dead Cell	filename.NEX.FCS
Cell Cycle	filename.CCY.FCS

Spreadsheet File

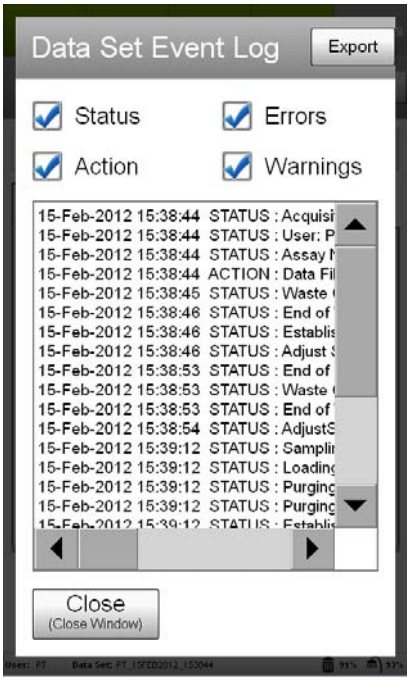
A spreadsheet file containing the data for all samples within a data set is automatically saved along with the FCS file in the same directory. The spreadsheet file contains a summary of the statistical results, sample information, and instrument settings for each sample run within a data set. It can be opened and analyzed using a spreadsheet program such as Microsoft Excel. The same file name assigned to the FCS data file is also used for the spreadsheet file, except it has the extension .CSV appended to the file name.

Event Log

Each time you run an assay, the system saves a log containing a list of all events that occurred during the assay. This information is contained within the FCS data file. To view this list of events, select **Options** from the Results screen for the assay, then select **Event Log**.

■ **NOTE:** There is also an event log for the System Check procedure and the Complete System Clean procedure. For information on those event logs, refer to the System Check and Cleaning chapters, respectively.

You can filter the list to view statuses, actions, errors, and/or warnings. Select the appropriate check box(es) to display the types of events you wish to view.



Example of Count & Viability event log

If errors or warnings occur during a run, a message appears in red below the plots, indicating that errors/warnings have been logged.

Errors

System Check:

- System Check failed due to avg MFI for [channel] – actual [x], Target value = [y]
  System Check failed due to percent CV for [channel] –value = [x]

Warnings

System Check:

- Loader arm is down.
- Concentration failed for replicate [1/2/3]
- Avg conc failed
- %CV failed

All assays:

- Sample Loader is down. Please raise the Loader arm.
- Adjust Settings timed out. Please re-enter Adjust Settings if necessary to complete the instrument set-up. (not applicable for System Check)
- The run timed out before enough events were acquired.

Annexin V and Count & Viability

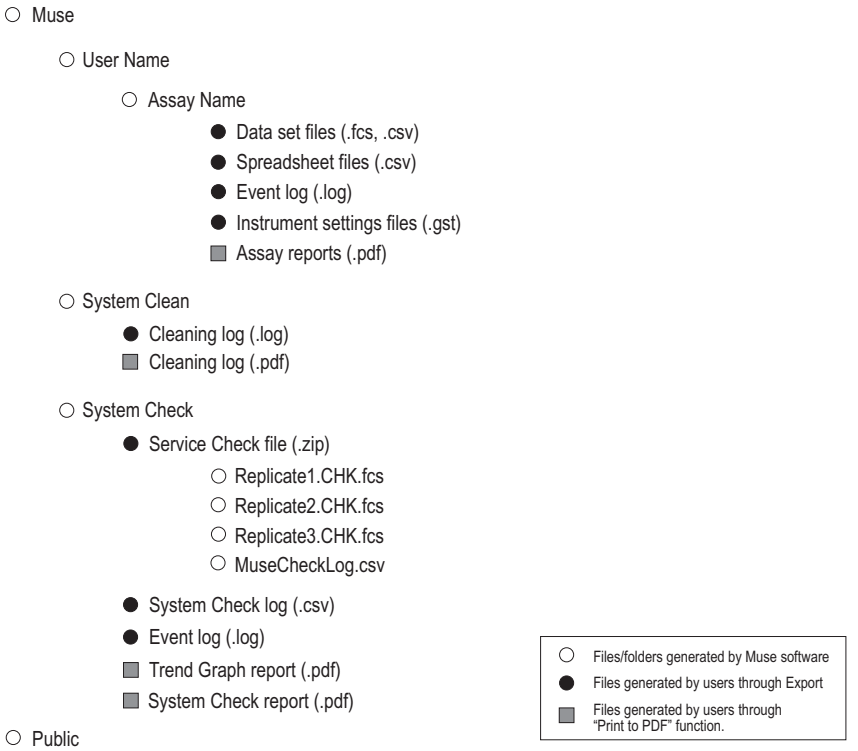
- More than 500 particles/µL. Sample is too concentrated. Please dilute or accuracy may be compromised.
- Less than 10 particles/µL. Sample is too dilute. Accuracy may be compromised.

Cell Cycle:

- More than 1200 particles/µL. Sample is too concentrated. Please dilute or accuracy may be compromised.
- Less than 25 particles/µL. Sample is too dilute. Accuracy may be compromised.

Folder and File Structure

When you export data to a USB drive using any of the export features, a folder structure is created on the USB drive. The following diagram outlines this folder structure.

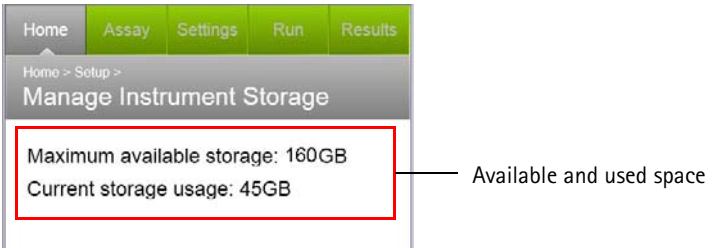


The Public folder is created automatically and allows administrators to share data. If an administrator wants other users to have access to certain data and/or instrument settings files, they can export the data to the Public folder. While operator-level users can access this data, they cannot modify the files or save files to the Public folder.

■ **NOTE:** The System Check and System Clean folders are created only if a user exports System Check and System Clean files.

File Storage

The Muse™ System has an internal hard drive for saving data files. To see the available storage, select **Setup** from the main menu, then select **Manage Instrument Storage**. The amount of available storage is displayed, as well as the amount of storage being used. For more information on managing instrument storage, see "Managing Instrument Data Storage" on page 86.



Appending Files

You can open an existing data set and append data to this file.

1 At the start of the assay select **View Results** from the assay screen. The Retrieve Data Set screen appears.

2 Select the location where the file is located. Keep in mind that you will not have access to the files from other users unless the files were exported to the Public folder.

3 Use the Filter field to sort by the file name or any part of the name. Or, use the calendar to search by date. Touch the calendar to open it and select the date.

4 Select the file and then select **Retrieve**.

Instrument Settings

The system allows you to save instrument settings files. These files contain instrument settings, as well as gates and marker settings. The extension .GST is appended to the file name.

You can retrieve these files from the MUSE directory, the Public folder, or USB drive when you start an assay. If you are an operator-level user, you can only access instrument settings from your own data folders in the Muse directory and USB drive.

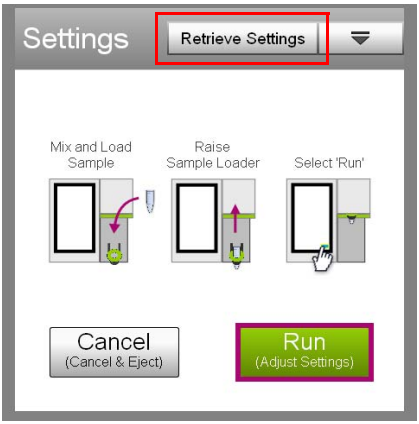
Assay	**Instrument Settings File Name**
Count & Viability	filename.VIA.GST
Annexin V & Dead Cell	filename.NEX.GST
Cell Cycle	filename.CCY.GST

You can recall this file later to:

- download the instrument settings to the instrument for acquisition
- apply the gates and markers to data during acquisition

Retrieving Instrument Settings

1 From the Settings screen at the start of a run, select **Retrieve Settings**.



2 Select the location where the settings file is stored.

3 Select the file and then select **Retrieve**.

You can filter the list by date. Select the calendar and choose the date when the settings were saved. Or, if you know the name of the file, enter any part of it in the Filter text field.

The settings are downloaded to the instrument. The system prompts you to run the adjust settings step.

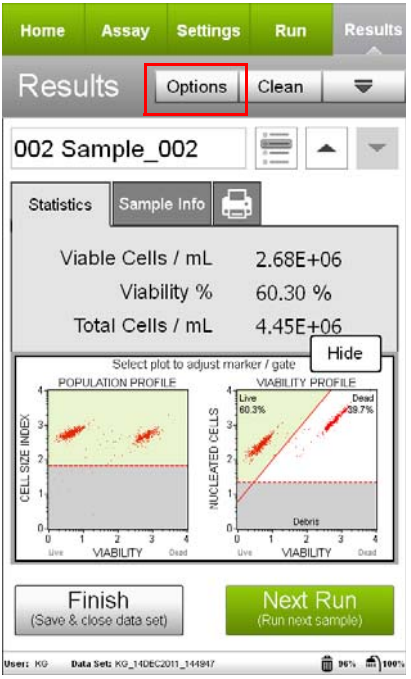
- Select **NO** if you wish to skip the adjust settings step and use the settings you just retrieved to run the assay. This will advance you to the Sample Info screen in preparation to run the first sample.
- Select **YES** if you wish to perform the adjust settings step using the settings you just retrieved. This will help to ensure that the retrieved settings are correct.

Saving Instrument Settings

1. Select **Options** from the Results screen after the sample is acquired.
2. Select **Save Current Settings**.
3. Select the location where you want to save the file.
   The default directory is the MUSE directory on the unit's hard drive. If you want to save the file to a USB drive, select the USB drive from the Save In field. A new directory will be created on the USB drive (MUSE\user name\assay name\instrument settings file).
4. Enter a file name or use the default file name.
5. Select **Save**.

Assay Overview

Detailed steps for each assay are covered in the reagent kit user's guide which can be found at www.millipore.com/muse. Some additional information on various tasks that can be performed from the assay screens is listed in the following section.

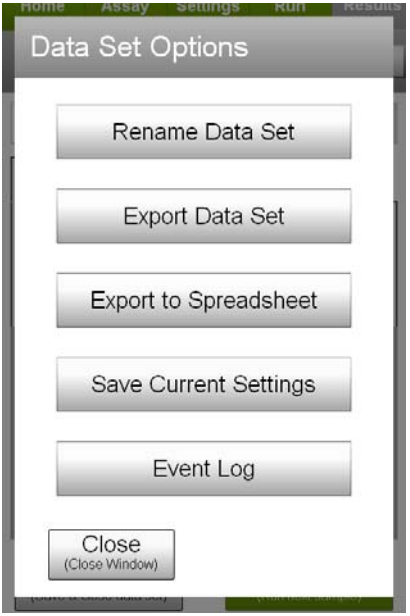


Example of Count & Viability results screen

Data Set Options

Use the **Options** button on the Results screen to access features that allow you to rename the data set, export data, save instrument settings, and view the event log.

1 Select **Options** in the title bar of the results screen.
The Data Set Options screen appears.



- To rename a data set, select **Rename Data Set**, edit the file name, and select **Apply**. You can also rename the data set at the completion of the run when you select **Finish**.
- To export the data set, select **Export Data Set**, select the USB drive from the Export Location field, enter a file name or leave the default. Select **Export**. You may want to export the data set so that you can share the data with other users.
- To export the data to a spreadsheet file, select **Export to Spreadsheet**, select the USB drive from the Export Location field, enter a file name or leave the default. Select **Export**.
- To save the current instrument settings, see "Saving Instrument Settings" on page 29.
- To view or export the event log, see "Event Log" on page 23.

About Dialog

The About dialog provides the software version number and copyright information, as well as technical support information. To access the About dialog touch the title bar of the main menu or any assay screen.

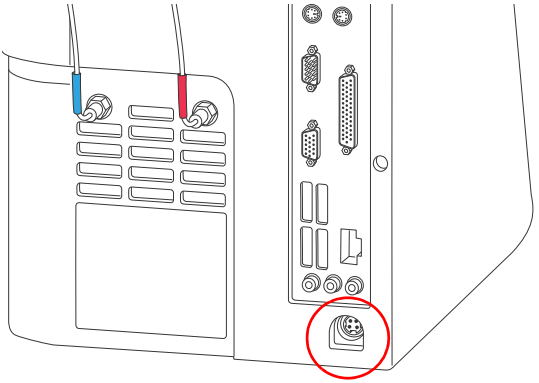
Touch the title bar to open the About dialog.

To prepare the system for use perform the following steps each day that you use the instrument.

- Empty the waste bottle and add bleach. See "Emptying the Waste Bottle" on page 71.
- Fill the cleaning solution bottle with ICF. See "Filling the Cleaning Solution Bottle" on page 70.
- Reset the fluid levels. See "Resetting Fluid Levels" on page 69.
- Perform a Complete System Clean. See "Performing a Complete System Clean" on page 61.

Turning On the Unit

1 Ensure the instrument is plugged in.



■ **NOTE:** If you are plugging in the instrument for the first time, it will automatically turn on. After a brief startup sequence, the logon screen appears. Refer to "Logging On as the First User" on page 35.

2 Press the power button located on the front of the unit below the touch screen.

A screen appears allowing you to select your user name from a list. Refer to "Logging On Once Users are Added" on page 38.

3 Fill the cleaning solution bottle to the fill line with Guava ICF. Empty the waste solution bottle, then add bleach to the first fill line (approximately 10 mL). See "Filling the Cleaning Solution Bottle" on page 70 and "Emptying the Waste Bottle" on page 71 for details.

4 Whenever you fill the cleaning solution bottle and empty the waste bottle, you will need to reset the fluid levels. Resetting the fluid levels sets the fluid indicators in the status bar to 100% and 100%. See "Resetting Fluid Levels" on page 69 for information.

5 Run a Complete System Clean at the start of each day to prime the fluid system. The first time you set up and use the instrument, perform two Complete System Clean procedures to ensure that the fluid system is sufficiently primed. See "Performing a Complete System Clean" on page 61.

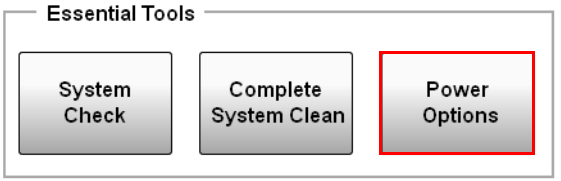
Turning Off the Unit

1 Run the Complete System Clean procedure at the end of the day before shutting down the unit. See "Performing a Complete System Clean" on page 61 for details.

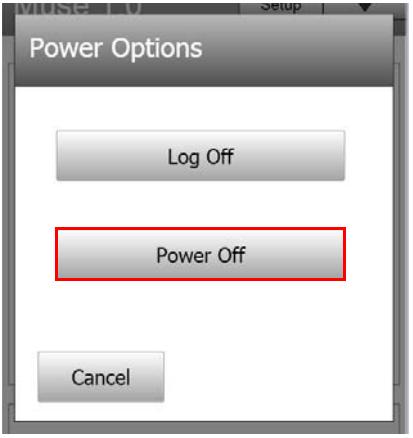
2 Leave the tube of DI water on the sample loader.

▲ **WARNING:** Do not leave a tube of Guava ICF, bleach, or any other cleaning agent loaded on the instrument overnight or for an extended period of time. Prolonged exposure to strong oxidizing agents will damage the flow cell. Always leave a fresh tube of DI water on the system when shutting it down. Change the tube of water regularly to ensure it is clean and free of particles.

3 Select **Power Options** from the main menu under Essential Tools.



4 Select **Power Off** to turn off the system.



Logging Onto the System

The system allows two levels of users—administrator and operator. For information on the different user levels and access privileges, see "Users & Access Levels" on page 79.

Logging On as the First User

The first time you log onto the system, you will be prompted to select **Administrator** or **Guest**. If you select Administrator you will have administrator privileges. If you select Guest you will have operator privileges.

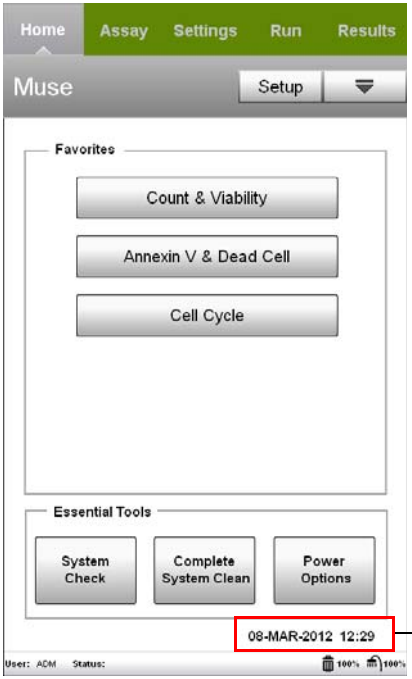
1 To add new users and customize the system, select **Administrator**.

- **NOTE:** Select **Guest** if you do not have a user account but want to use the instrument. Files will be saved with GST initials.
- **IMPORTANT NOTE:** Once you log on as an administrator, it is a good idea to add yourself as a user with administrator-level access. When you add a new administrator-level user, the default Administrator user (shown below) will be overwritten. For this reason, we do not recommended running assays and acquiring data

sets as a default Administrator, as the data will no longer be accessible.

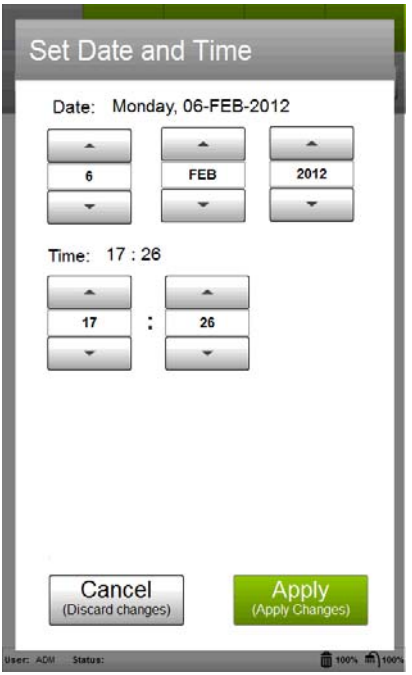
Default Administrator and Guest users

2 The main menu appears. Touch the date and time located in the lower-right corner of the screen.



Set date and time.

3 Use the arrow buttons above and below each field to select the day, month, year, hours, and minutes.



4 Select **Apply**.

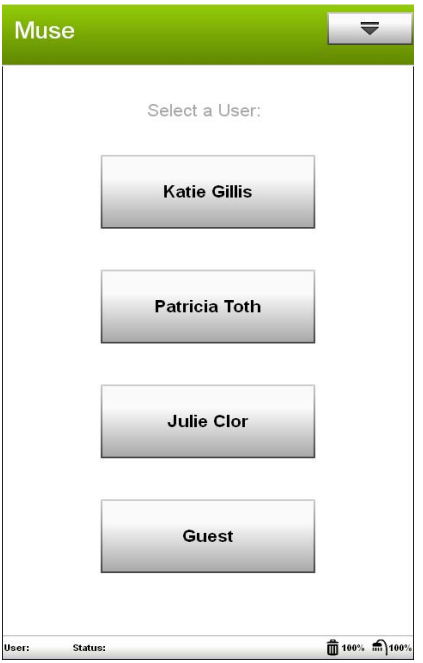
5 See "Adding a New User" on page 80 for information on adding new users.

Logging On Once Users are Added

When you start the system, a screen appears allowing you to select your user name from a list.

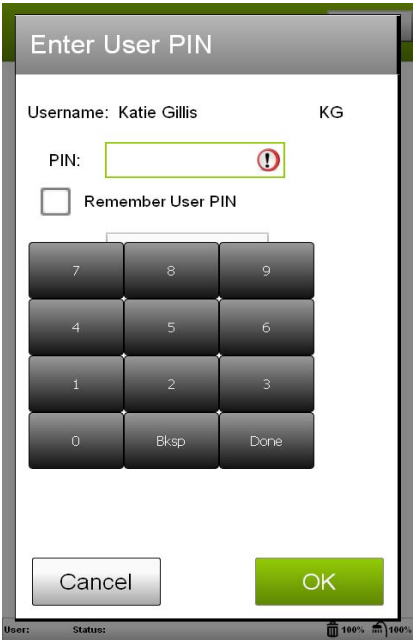
1 Select your name from the list to display the main menu.

If your name does not appear, contact your system administrator.



2 Enter your PIN and press **OK**.

If security is not an issue and you choose not to enter a PIN each time you log in, you can select **Remember User PIN**. The next time you log in, simply select **OK** to accept the PIN.



The main menu appears after your PIN is correctly entered.

Forgetting Your User PIN

If you forget your PIN, your administrator can reset it by assigning you a new PIN. If you are an administrator and you forget your PIN, another administrator can reset it. If you are the sole administrator and you forget your pin, you will no longer have administrator access to the system and therefore cannot perform administrator functions. For this reason, it is a good idea to save your PIN in a secure place.

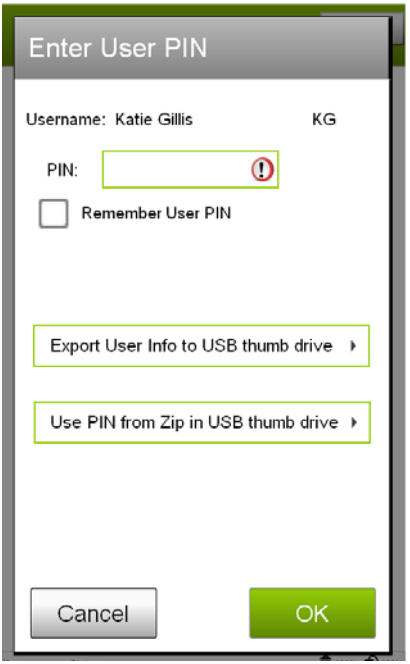
If you are the sole administrator and you forget your PIN, you can export your user information to a USB drive and send it to EMD Millipore. We will send a PIN back to you, which you can then import.

1 If you forgot your PIN, select **Done** or touch outside the keypad to close it, then select **Forgot User PIN**.

2 Insert a USB drive.

3 Select **Export User Info to USB thumb drive.**

- **NOTE:** Be aware that once you export your PIN you will no longer be able to log onto the system even if you should remember your PIN.



4 Select the drive you want to export to, then select **Save**.

5 Contact Tech Support. You will be asked to e-mail the zip file from your thumb drive to Tech Support.

6 Tech Support will e-mail you another file which you will save to a USB drive.

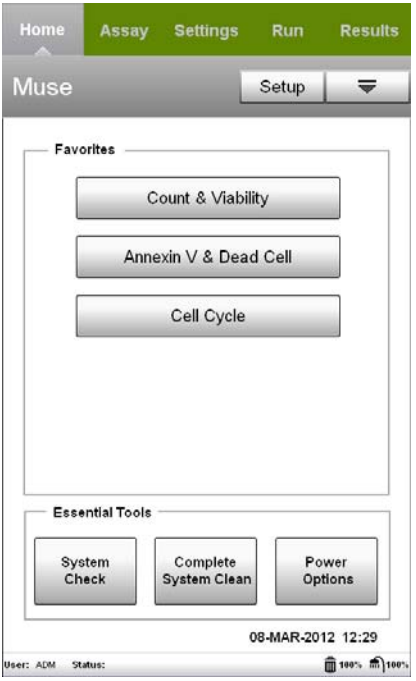
7 Insert the USB with the file from Tech Support. Begin to log on—select your user name and **Forgot User PIN**. Then select **Use PIN from Zip in USB thumb drive**.

8 Follow the steps to reset the PIN.

Selecting an Assay to Run

The main menu displays the following assays. Select the assay you wish to run.

- Count & Viability
- Annexin V & Dead Cell
- Cell Cycle



For information on performing a System Check, see "Running a System Check Procedure" on page 43. For information on running an assay, refer to the kit user's guide, which can be found at www.millipore.com/muse.

System Check

The System Check procedure is used to verify the performance of your Muse™ System by assessing counting accuracy and fluorescence detection. The Muse™ System Check Kit contains Muse™ System Check Beads and Muse™ System Check Diluent. Perform the System Check procedure daily before running any of the assays to ensure that the instrument provides reliable, accurate results.

Running a System Check Procedure

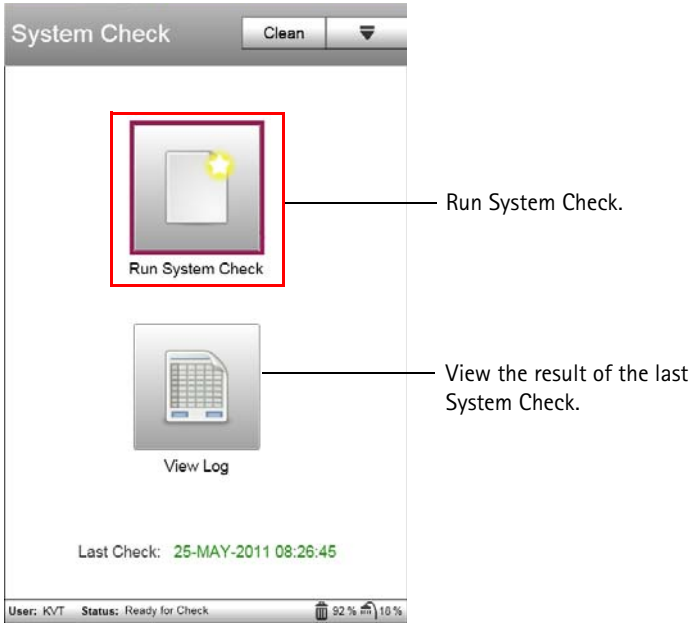
Run a System Check at the start of each day that you use the instrument to ensure that it is performing properly. Three replicates of the System Check Bead sample are acquired. The results are averaged to determine if they are within the expected range.

Run a Complete System Clean at the start of each day and before performing the System Check procedure. A cleaning cycle will prime the fluid system and remove bubbles that may have formed in the tubing. See "Performing a Complete System Clean" on page 61 for details.

1. Prepare a 1:20 dilution of System Check Beads. Refer to the *Muse™ System Check Kit User's Guide* for information.
2. Select **System Check** under Essential Tools at the main menu to display the System Check screen.
3. A message appears prompting you to check the fluid levels in the cleaning and waste bottles. Check the fluids, then click **Close**.

   Always remember to reset the fluid levels when you fill the cleaning solution bottle and empty the waste bottle. For information on resetting the fluid levels, see "Resetting Fluid Levels" on page 69.

4 Select **Run System Check.**



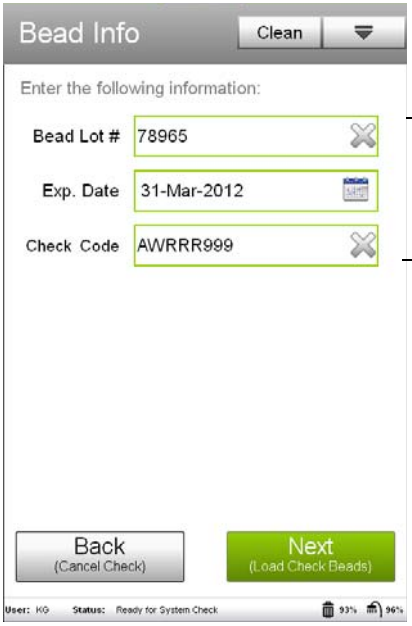
5 The first time you run the procedure, enter the bead lot number, expiration date, and check code.

- Enter the Bead Lot # and press **Done** on the keypad.
- Touch the calendar icon in the Exp. Date field to select the expiration date. Touch outside the calendar to close it.
- Touch the Check Code field and enter the code.

All values are required and can be found on the information card that comes with the bead kit.

Once you enter this information, it will remain in the software. Each time you run the procedure, check the information to ensure it is accurate. Update the values when a new lot number of System Check Beads is used, if necessary.

6 Select **Next**.



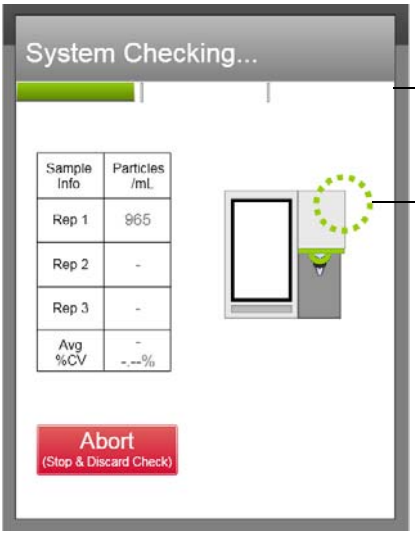
Touch a field to display a keypad allowing you to enter the values.

7 Mix the tube of prepared beads and load it on the system.

8 Select **Run**.

The system performs a prime, then acquires the first replicate. The progress bar and fly wheel provide indicators as to the status of acquisition. The progress bar is divided into three sections—one for each replicate. If the fly wheel is turning but the progress bar in not

advancing, the fluid system may be clogged or the beads may have settled to the bottom of the tube. If the beads settled, select **Abort**, unload the tube and mix. Then reload and select **Run System Check** again.

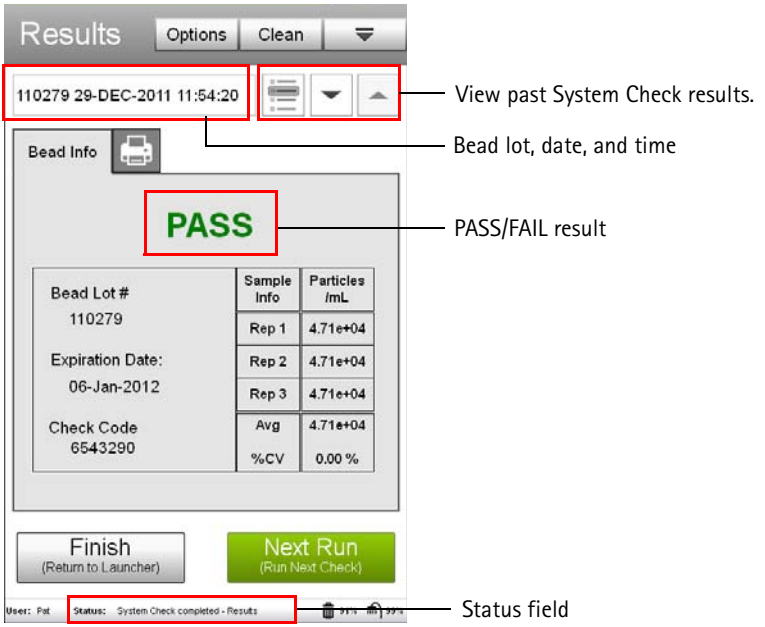


9 Remove the tube and vortex it to resuspend the beads.

10 Load the tube and select **Run**.
The system acquires the second replicate.

11 Repeat steps 9 and 10 to acquire the third replicate.

Upon completion, the system displays a PASS/FAIL result and the Particles/mL value for each replicate, as well as the average.



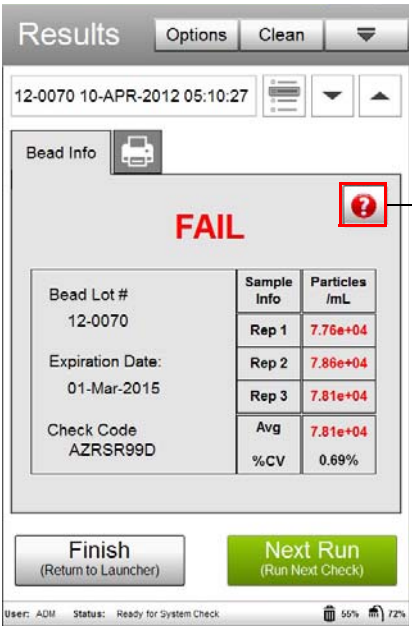
The bead lot number, date, and time of the run appears just above the Bead Info and printer tabs. All runs in the System Check log are displayed in this format. The Status field at the bottom of the screen indicates that the System Check was completed.

System Check Results

The result appears as PASS or FAIL. The bead count (Particles/mL) for each replicate and the average are displayed, as well as the %CV.

If any result for Particles/mL falls outside ±10% of the expected value, the result is outside the acceptable range and appears in red. If the procedure fails, touch the Help button (?) to display troubleshooting information.

- **NOTE:** The System Check procedure can also fail if the red and yellow mean fluorescence intensities (MFIs) fall outside the expected results. If all Particles/mL values are green, but the result is FAIL, contact EMD Millipore Technical Support.



Touch Help button to get troubleshooting information.

Printing Results

To print the results, select the print tab to the right of the Bead Info tab on the Results screen. You can choose to print the current results, the log list (see "Viewing Past System Check Results" on page 50), or a trend graph.

1 Select the printer from the drop-down list of printers.

The list displays all available printers. If no printers are detected, the default printer is **PDF to USB**, which allows you to export a PDF.

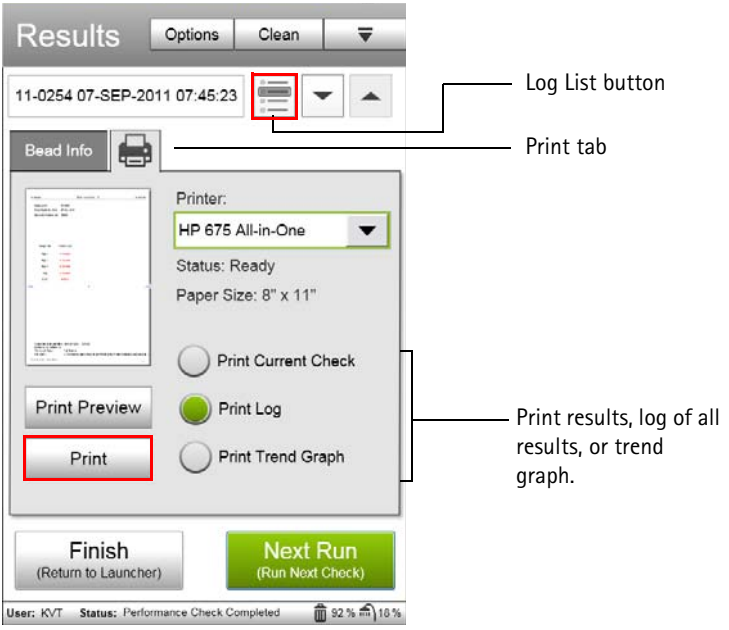
2 Select the **Print Current Check** button to print the current results.

You can also print the entire log list (past System Check results) or the trend graph. If you select to print the trend graph, the number of runs included in the printout is based on the number of runs selected at the Trend Graph screen (see "Viewing a Trend Graph" on page 53).

3 (Optional) Select **Print Preview** if you want to view the results as they will print.

4 Select **Print**.

If no printers are available or if you wish to export to a PDF, select **PDF to USB**, then select **Print**. The Export to PDF screen appears. Select the USB port, enter a file name for the report file, or leave the default name. Select **Export**.



Viewing Past System Check Results

To see the results from previous runs, select the Log List button below the Clean button on the Results screen. You can also use the arrows to the right of the Log List button to quickly scroll through and display previous System Check results in the Bead Info tab.

Each entry in the System Check Log represents one System Check run and includes the user who ran the test, the bead lot number, the date and time the test was run, and whether or not the test passed (Y or N). A hyphen indicates that the run was aborted. Use the arrow buttons at the bottom of the screen to scroll through the pages of results.

Select a run from the list to display the selected results in the Bead Info tab.

For information on clearing and exporting the System Check Log, or viewing a trend graph or the event log, see "System Check Options" starting on page 51.

System Check Options

Select **Options** at the top of the System Check Results screen to export or clear the System Check results log, export the Service Check file, or view the trend graph or event log.

Clearing the System Check Log

The System Check Log is a list of all past System Check results. After exporting the System Check Log, you may wish to delete all entries in the log. Once the data is cleared, you will no longer be able to view past results or generate trend graphs. Only administrator-level users can clear the System Check Log.

1 Select **Options** from the System Check Results screen.

2 Select **Clear Check Log**.

3 Select **Clear** to confirm.

Exporting the Check Log

The System Check Log is a list of all past System Check results. Only administrator-level users can export the System Check Log.

1 Select **Options** from the System Check Results screen.

2 Select **Export Check Log**.

3 Select the USB drive from the Export Location field.

4 Enter a name for the log file, or leave the default name.

5 Select **Export**.

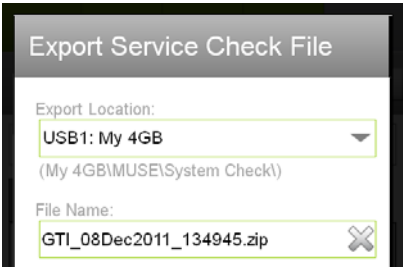
Exporting the Service Check File

The Service Check file is a zipped file containing the detailed results from the most recent System Check run. This file is used by service personnel to troubleshoot your system if your System Check results continue to fail. Use the Export Service Check File feature to export the file so that you can send it to EMD Millipore.

1 Select **Options** from the System Check Results screen.

2 Select **Export Service Check File**.

3 Select the USB drive from the Export Location field.



4 Enter a name for the log file, or leave the default name.

5 Select **Export**.

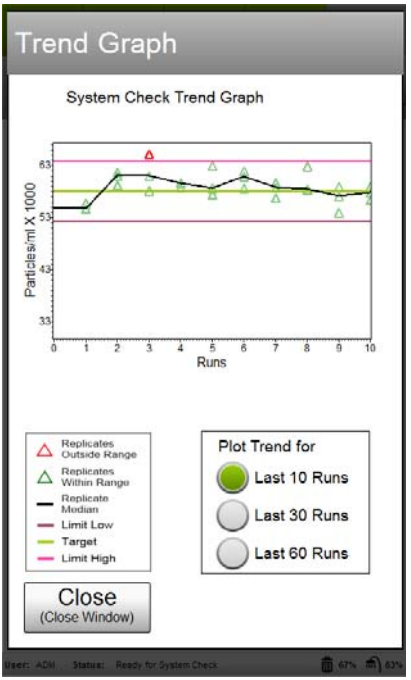
Viewing a Trend Graph

You can view a trend graph of the particles/mL data from the last 10, 30, or 60 System Check runs. A data point appears for each of the three replicate values.

1 Select **Options** from the System Check Results screen.

2 Select **View Trend Graph**.

3 Choose the number of runs (10, 30, or 60) that you wish to plot.



A legend in the lower-left corner lists the information found on the graph. A description of each item in the legend appears in the following table.

Legend Item	Description
Replicates Outside Range	Data point appears as a red triangle (value falls outside the high or low 10% limit lines)
Replicates Within Range	Data point appears as a green triangle
Replicate Median	A black line connects the median values from each triangle

Legend Item	Description
Limit Low	A purple line appears 10% below the expected particle count determined by the Check Code
Target	A green line at the expected particle count determined by the Check Code
Limit High	A pink line appears 10% above the expected particle count determined by the Check Code

4 (Optional) You cannot export the trend graph to a CSV file, but you can print to a pdf.

- Select the number of runs (last 10, 30, or 60) you wish to include from the Trend Graph screen, then close the screen. Close the System Check Log Options screen.
- Select the printer tab from the System Check Results screen.
- Select **Print Trend Graph** (see page 49), then select **PDF to USB** from the Printer list, and finally select **Print**.

Viewing the System Check Event Log

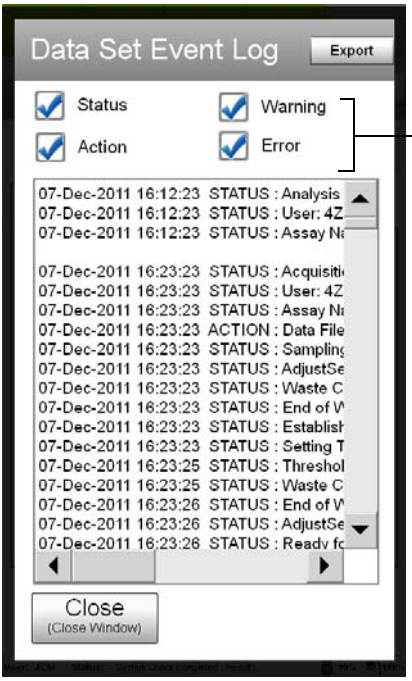
The system saves a list of all events that occurred during the System Check run to a log file.

1 Select **Options** from the System Check Results screen.

2 Select **View Event Log**.

The event log contains a list of all events that occurred during the System Check run. Every step that the operator performed and every step that the instrument performed, independent of the operator, are logged, as well as warnings and errors that occurred during the run.

3 You can filter the list to view statuses, actions, errors, and/or warnings. Select the appropriate check box(es) to display the types of events you wish to view.



Filter the event log list by selecting the types of events you wish to view.

If errors or warnings occur during a run, a message appears in red on the results screen, indicating that errors/warnings have been logged. For a list of System Check errors and warnings, see page 23.

Exporting the Event Log

You can export the System Check event log to a CSV file. If you are currently viewing the event log, proceed to step 3.

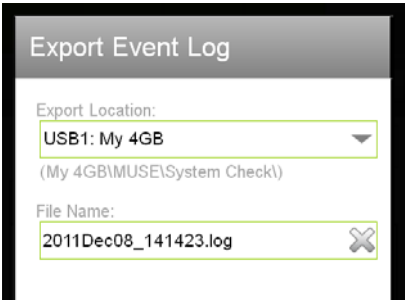
The entire event log is exported regardless of whether you filtered the list.

1 Select **Options** from the System Check Results screen.

2 Select **View Event Log**.

3 Select **Export** from the Data Set Event Log screen.

4 Select the USB drive from the Export Location field.



5 Enter a name for the log file, or leave the default name.

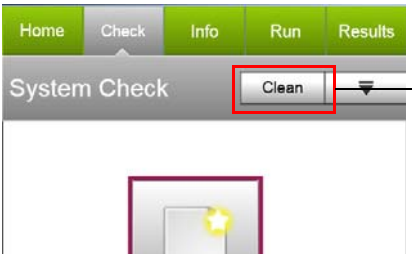
6 Select **Export**.

Cleaning and Maintenance

The system's sampling precision depends on the integrity of the fluid pathway; therefore, it is important to maintain a clean system. Do not allow samples to remain loaded on the instrument for extended periods of time, as they may eventually clog the fluid system. Perform frequent cleaning cycles to prevent the build-up of cellular debris that may restrict sample flow.

Cleaning Protocols

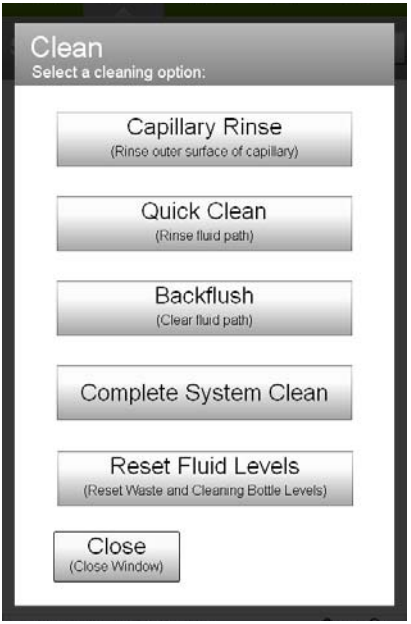
Several cleaning options are available depending on the type of cleaning you wish to do. The following protocols are available by selecting **Clean** from any assay screen, as well as the System Check screen (shown below).



Select Clean from System Check or any assay screen to access the cleaning options.

- **Capillary Rinse** allows you to rinse the outer surface of the capillary. This can be helpful if you want to reduce carry-over between samples, or when cell counts are critical.
- **Quick Clean** is a short cleaning cycle that allows you to clean the system during and after an assay, or as often as you like throughout the day.
- **Backflush** reverses fluid out of the capillary. Use this feature if you suspect a clog. Always load a tube of 20% bleach when you perform a backflush.
- **Complete System Clean** allows you to thoroughly clean the instrument at the end of the day or between assays, if needed.

- **Reset Fluid Levels** sets the fluid level status for the waste bottle and cleaning solution bottle back to 100% and 100%, after you have emptied the waste bottle and filled the cleaning solution bottle.



Cleaning options

Cleaning the Outside of the Unit

Clean the outside of the instrument by wiping it down with a soft cloth moistened with a 70% isopropyl alcohol or a 10% bleach solution in DI water. Follow with a cloth moistened with water. To avoid getting excess liquid on the instrument, do not spray these solutions on the instrument.

To clean the touch screen, wipe it with 70% isopropyl alcohol.

▲ **WARNING:** To avoid exposure to laser radiation or electric shock, do not open the unit or attempt to perform any internal maintenance. There are no user-serviceable parts.

Rinsing the Capillary

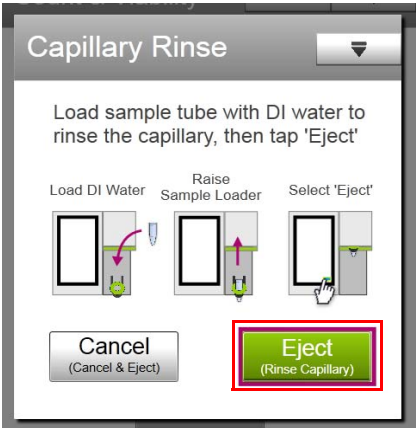
Capillary Rinse allows you to rinse the outside of the capillary by simply loading and unloading a tube of water. Use this option to avoid carryover.

1 Select **Clean** from the System Check screen or any assay screen.

2 Select **Capillary Rinse** from the list of cleaning protocols.

3 Load a full tube of DI water on the unit.

4 Select **Eject**.



5 Discard the tube of water.

Running Quick Clean

Quick Clean is a short cleaning cycle that cleans the fluid system during and after an assay, or as often as you like throughout the day. If you are running lots of samples with high background, run Quick Clean according to the frequency recommended for the assay—a Recommended Quick Clean message will appear for every N number of samples.

1 Select **Clean** from the System Check screen or any assay screen.

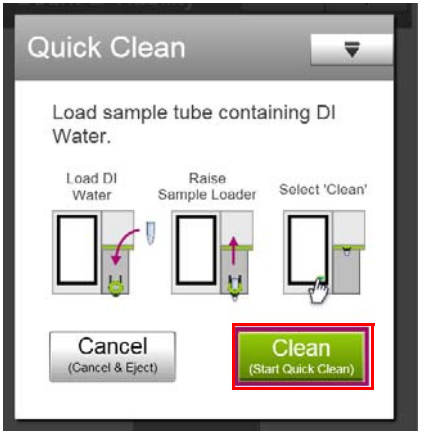
2 Select **Quick Clean** from the list of cleaning protocols.

3 The system prompts you for ICF, however, you can load a tube containing any one of the following solutions:

- DI water to quickly flush out the system.
- Guava ICF to clean the system (follow with a second Quick Clean using water to rinse)

- 10% bleach solution in Guava ICF (1 part bleach in 9 parts Guava ICF) [follow with a second Quick Clean using water to rinse]

4 Select **Clean** to run the solution.



5 If you used water you are done with the Quick Clean procedure. If you used Guava ICF, either straight or with bleach, you will need to run water to rinse the fluid system. Select **Quick Clean** from the cleaning protocol screen. Load a full tube of DI water, then select **Clean**.

You may continue running samples, or leave the tube of water loaded on the unit until you are ready to use the system again.

Backflushing the Capillary

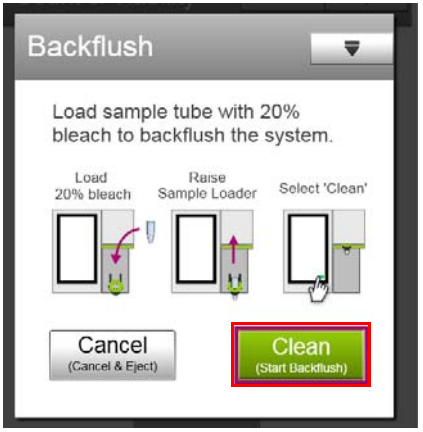
The Backflush feature reverses the flow of fluid out of the capillary. Use this feature when you suspect that the fluid pathway is clogged.

1 If you suspect a clog during acquisition, select **Clean** from the System Check screen or any assay screen.

2 Select **Backflush** from the list of cleaning protocols.

3 Load a tube containing 100 µL of 20% bleach.

4 Select **Clean**.



5 When the backflush is complete, throw out the tube of bleach you used in step 3, as it may contain debris from the backflush.

6 Select **Quick Clean** to rinse the residual bleach from the capillary. Load a full tube of DI water on the unit and select **Clean**.

7 Replace the sample and continue with the assay or procedure you were running.

Performing a Complete System Clean

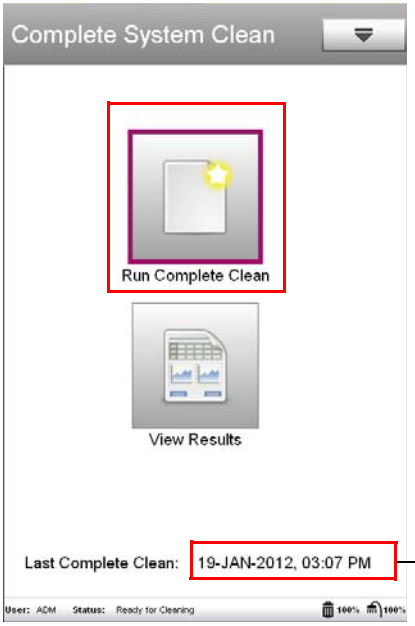
Run the Complete System Clean feature to clean the unit at the end of each day, or between assays if a thorough cleaning is needed. You can also run this cleaning cycle to prime the fluid system or if you suspect there is air in the fluid lines. The first time you use the instrument, perform two Complete System Clean procedures.

Always ensure the cleaning solution bottle is filled with ICF whenever you run the Complete System Clean.

1 Select **Complete System Clean** under Essential Tools at the main menu.

This feature is also available by selecting **Clean** from the System Check screen or any assay screen, then selecting **Complete System Clean** from the list of cleaning protocols.

2 Select **Run Complete Clean**.

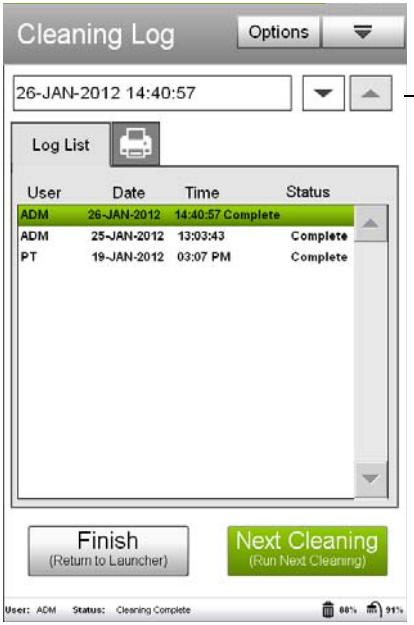


Displays the last time a complete system clean was performed.

3 Load a full tube of Guava ICF on the unit and select **Run**.

4 When the ICF cycle is complete, the system prompts you for a tube of DI water. Load a full tube of DI water on the unit and select **Continue**.

When the system cleaning procedure is complete, the Cleaning Log appears. Each entry in the log represents one System Cleaning procedure. The list shows the user who performed the cleaning, the date and time the cleaning was performed, and whether the cleaning was completed or aborted.



Scroll through cleaning log entries.

5 If you are shutting down the system, leave the tube of DI water used for cleaning loaded on the unit and select **Finish** to return to the main menu.

▲ **WARNING:** Do not leave a tube of Guava ICF, bleach, or any other cleaning agent loaded on the instrument overnight or for an extended period of time. Prolonged exposure to strong oxidizing agents will damage the flow cell. Always leave a tube of DI water on the system after cleaning and when shutting down. Change the tube of water regularly to ensure it is clean and free of particles.

6 (Optional) To run the cleaning procedure again, select **Next Cleaning**.

Printing the Cleaning Log

You can print a list of all Complete System Clean procedures performed since the last time the cleaning log was cleared. The list contains the date and time that each Complete System Clean was run, as well as the operator who performed the cleaning procedure and whether the cleaning was completed or aborted.

1 Select the printer tab from the Cleaning Log screen.

If you did not just complete the system cleaning procedure, select **Complete System Clean** under Essential Tools at the main menu, then select **View Log/Results** from the Complete System Clean screen.

2 (Optional) Select **Print Preview** to see the list as it will print.

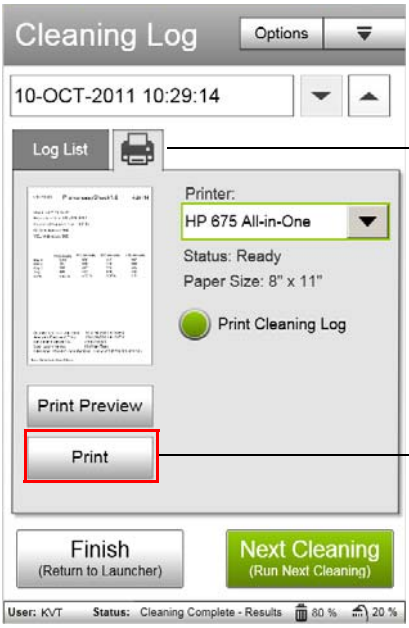
3 If necessary, select the printer from the Printer drop-down list.

If a printer is detected, it will appear as the default. If no printers are available, the default is **PDF to USB**, allowing you to save the results to a PDF file.

4 Select **Print** to print the list.

If no printers are available or if you wish to export to a PDF, select **PDF to USB**, then select **Print**. The Export to PDF screen appears. Select the

USB port, enter a file name for the report file, or leave the default name. Select **Export**.



Cleaning Log Options

You can view the cleaning log, and clear or export the entries in the log list. While only administrator-level users can clear the log, any user can export the log.

1 Select **Options** from the Cleaning Log screen.

If you did not just complete the system cleaning procedure, select **Complete System Clean** under Essential Tools at the main menu, then

select **View Log/Results** from the Complete System Clean screen, then **Options**.

2 Refer to the following sections for information on the task you wish to perform.

Clearing the Cleaning Log

After you export the Cleaning Log, you can clear the log. Only administrator-level users can clear the Cleaning Log.

1 Select **Options** from the Cleaning Log screen.

2 Select **Clear Cleaning Log** from the Cleaning Log Options screen.
A message appears informing you that the action cannot be undone.

3 Select **Clear** to confirm and clear the log.

Exporting the Cleaning Log

You can export the cleaning log to a CSV file. The file contains a list of all Complete System Clean procedures performed since the last time the cleaning log was cleared. The list includes the dates and times that each Complete System Clean was run, as well as the operators who performed the cleaning procedure and whether the cleaning was completed or aborted.

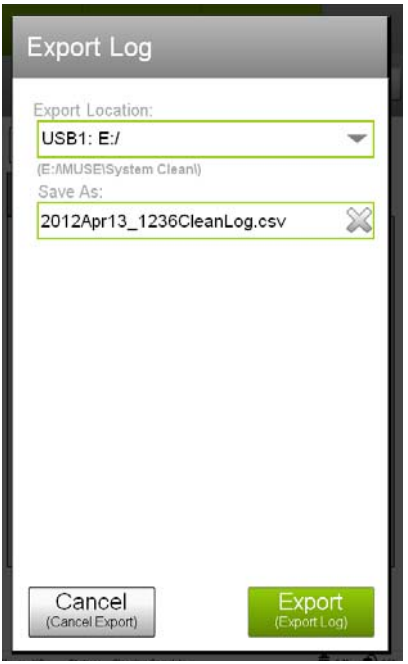
1 Select **Options** from the Cleaning Log screen.

2 Select **Export Cleaning Log** from the Cleaning Log Options screen.

3 Select the USB drive from the Export Location field.

4 Enter a file name or use the default file name.

5 Select **Export.**



Viewing and Exporting the Cleaning Event Log

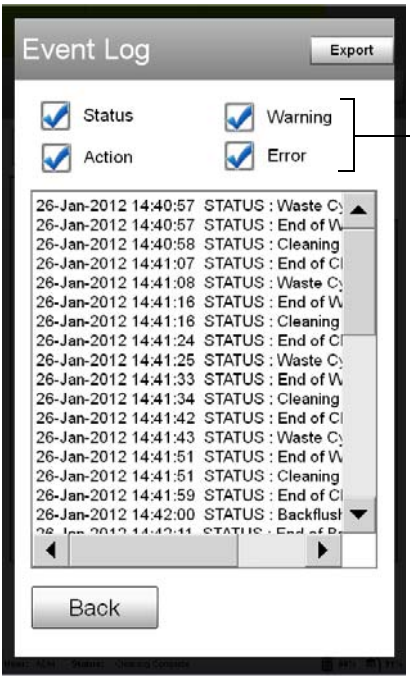
The system keeps track of and saves a list of all events that occurred during the Complete System Clean procedure to a log file.

1 Select **Options** from the Cleaning Log screen.

1 Select **Export Event Log** from the Cleaning Log Options screen.

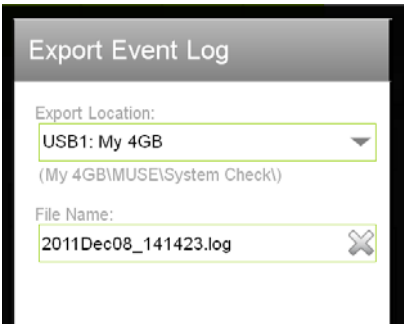
The event log contains a list of all events that occurred during the Complete System Clean. Every step that the operator performed and every step that the instrument performed during the procedure are logged.

2 You can filter the list to view statuses, actions, errors, and/or warnings. Select the appropriate check box(es) to display the types of events you wish to view.



Filter the event log list by selecting the types of events you wish to view.

3 (Optional) If you wish to export the cleaning event log, select **Export** from the Event Log screen, select the USB drive from the Export Location field, enter a file name or use the default name, and select **Export**.



Resetting Fluid Levels

Use the Reset Fluid Levels option to reset the status indicator for the waste bottle and cleaning solution bottle back to 100% and 100% after you have emptied the waste bottle and filled the cleaning solution bottle. It is important to reset the fluid levels each time you fill and empty the bottles so that the instrument can accurately determine the amount of fluid in each bottle.

When the waste bottle has 1% capacity remaining or the cleaning solution bottle has 1% fluid remaining, a message appears prompting you to fill the cleaning solution bottle and empty the waste bottle.

1 Select **Clean** from the System Check screen or any assay screen.

2 Select **Reset Fluid Levels** from the list of cleaning protocols.
You can also select **Complete System Clean** under Essential Tools at the main menu, then select **View Log/Results**, then **Options**.

3 Select **Next**.
The levels are now set to 100% for both the cleaning and waste bottles.

Filling the Cleaning Solution Bottle

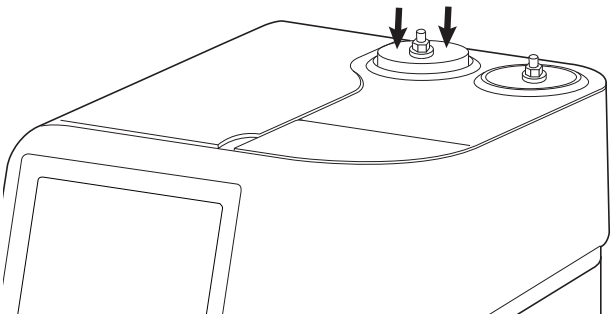
Fill the cleaning solution bottle with Guava ICF at the start of each day before you run the Complete System Clean procedure, and as needed. Do not allow the bottle to empty. This will pull air into the fluid system and require that you prime the system with water.

The status bar located at the bottom of the screen indicates the amount of cleaning solution left in the bottle. When the remaining volume reaches 20%, the value appears in red, indicating it is time to fill the bottle with ICF. When the volume reaches 1%, a message appears indicating that you need to *immediately* fill the cleaning solution bottle and empty the waste bottle before proceeding.

Example shows cleaning solution bottle has 20% solution remaining (80% has been used).

1 Uncrew the tubing from the top of the cleaning solution bottle.

2 Press down on the cleaning solution bottle to release it from the unit. The bottle will pop up slightly allowing you to remove it.



Push down on the bottle to remove it.

3 Unscrew the cap.

4 Fill the bottle to the fill line with Guava ICF.

5 Replace the cap and reinstall the bottle in the instrument.
Align the shower icon on the bottle with the notch/line on the instrument. Press down on the bottle to engage it.

6 Reconnect the fluid line to the top of the bottle.

Emptying the Waste Bottle

Empty the waste bottle at the start of each day before you run the Complete System Clean procedure, and as needed.

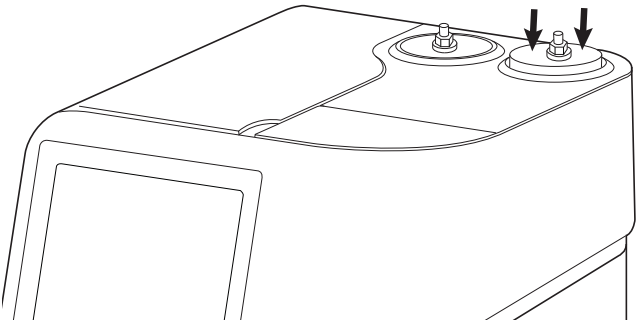
The status bar located at the bottom of the screen, indicates the amount of space left in the waste bottle. When the remaining space reaches 20%, the value appears in red, indicating it is time to empty the waste bottle. When the remaining space reaches 1%, a message appears indicating that you need to *immediately* empty the waste bottle and fill the cleaning solution bottle before proceeding.

Example shows waste bottle has 20% capacity remaining (80% full).

▲ **WARNING:** Handle all biological specimens and materials they come in contact with as if capable of transmitting infection. Dispose of these materials using proper precautions in accordance with federal, state, and local regulations.

1 Unscrew the tubing from the top of the waste bottle.

2 Press down on the waste bottle to release it from the unit. The bottle will pop up slightly allowing you to remove it.



Push down on the bottle to remove it.

3 Carefully unscrew the cap.

4 Empty the contents according to your local and state biohazardous waste disposal guidelines.

5 Rinse the bottle with water.

6 Add approximately 10 mL of bleach to the empty waste bottle.

7 Replace the cap and reinstall the bottle in the instrument.

Align the trash can icon on the bottle with the notch/line on the instrument. Press down on the bottle to engage it.

Reconnect the fluid line to the top of the bottle.

Cleaning the Capillary

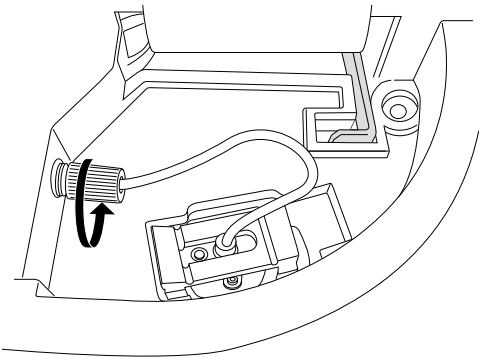
Use the flow cell cleaning tool (syringe) to clean the flow cell.

▲ **WARNING:** To avoid exposure to laser radiation, turn off the power to the Muse™ System before attempting to clean the capillary.

1 Use the eject button on the main menu or any assay screen to lower the sample loader arm.

2 Open the flow cell access door at the top of the instrument.

3 Use your fingers to unscrew the tubing from the instrument.

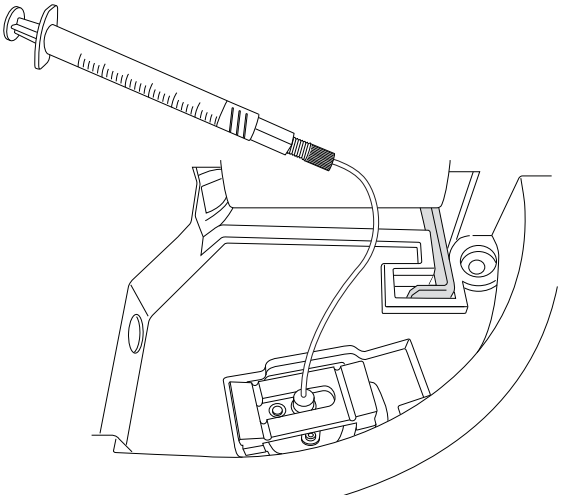


4 Fill the syringe with DI water or Guava ICF, then screw the syringe onto the tubing.

5 Apply gentle, steady pressure to the plunger and watch as the fluid flows from the tip of the capillary.

- Make sure the fluid stream is straight. If it is not straight, the tip of the capillary may be chipped or there may be a partial clog in the flow cell.

- Check the capillary to ensure there are no leaks along the length of the tube or liquid pooling at the top of the flow cell.



6 Unscrew the syringe tool from the tubing.

7 Reconnect the tubing to the instrument. Make sure the tubing is screwed on tightly and there are no constricting kinks or twists.

8 Close the access door.

9 Prime the system by running a Quick Clean procedure. Perform a System Check procedure.

Replacing the Flow Cell

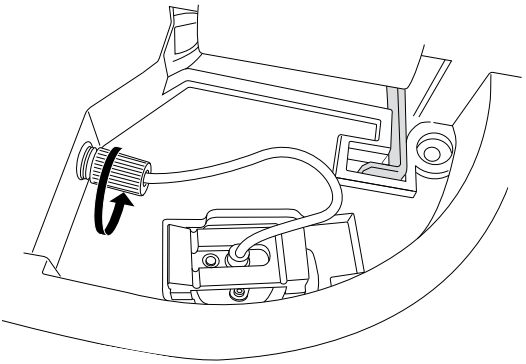
You can replace the flow cell if it becomes damaged or clogged so severely that backflushing and cleaning the system do not fix the problem. When removing and replacing the flow cell, handle it with care. The capillary tube is fragile; avoid touching it unnecessarily. Do not force the flow cell into the receptacle.

▲ **WARNING:** To avoid exposure to laser radiation, turn off the power to the Muse™ System before attempting to remove the flow cell.

1 Use the eject button on the main menu or any assay menu to lower the sample loader arm.

2 Open the flow cell access door at the top of the instrument.

3 Use your fingers to unscrew the tubing from the instrument.

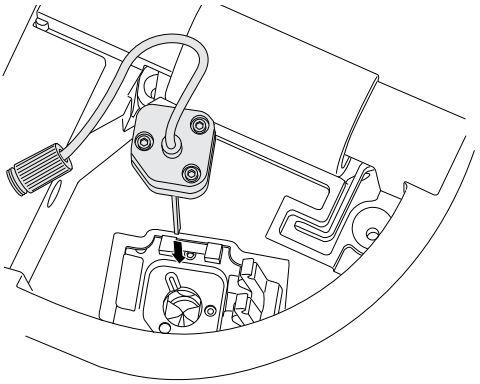


4 Release the clamp that holds the flow cell assembly in place. Press down on the left edge of the clamp to release the forked side on the right.

5 Holding onto the tubing as closely to the green connector as possible, gently pull up on the flow cell assembly to remove it from the receptacle. Keep the assembly completely vertical until the capillary is clear of the instrument.

6 Discard the flow cell and replace it with a new flow cell assembly.

7 Install the new flow cell. The flow cell fits only one way into the receptacle. Align the cut-out on one corner of the flow cell with the cut-out in the receptacle. Avoid bumping the capillary tube against the instrument or sides of the receptacle as you install it.



8 Close the clamp to secure the flow cell.

9 Reconnect the tubing to the instrument. Make sure the tubing is screwed on tightly and there are no constricting kinks or twists.

10 Close the access door.

11 To ensure that the flow cell was correctly installed, run Quick Clean to prime the system. Perform a System Check procedure.

Preparing the Unit for Depot Service

Contact EMD Millipore Technical Service for the Decontamination Form and instructions. The form must be returned via e-mail before you can return the instrument. A return authorization (RMA) number will be issued to you. Write this number on the outside of the shipping box.

If you did not save your original shipping box, EMD Millipore can send one to you.

1 Perform a Complete System Clean, then power off the system.

2 Empty and rinse out the waste and cleaning solution bottles and place them in their original box.

3 Wipe the outside of the instrument with 70% isopropyl alcohol or a 10% bleach solution in DI water, followed by water.

4 Open the flow cell access door and disconnect the fluid tubing from the instrument.

5 Release the clamp securing the flow cell and carefully remove the flow cell.

6 Use an empty syringe to flush the flow cell with air. Place the flow cell in its original box.

7 Lift the loader arm in the up position.

8 Place the shipping insert (with the foam attached) on a flat surface, then place the instrument in the foam cut-out.

The tall block of foam should be on the right, as it will be positioned under the loader arm when sides are folder up.

9 Fold the sides of the insert up, making sure the foam block fits under the loader arm.

10 Place the power supply and power cord behind the instrument.

11 Use both handles on the insert to lift the instrument and lower it into the shipping box.

12 Place the fluidics box (with flow cell, fluid bottles, and tubing) in the space in front of the instrument.

13 Ship the instrument to:

EMD Millipore

ATTN: Muse RMA (insert the RMA number issued to you)

25801 Industrial Blvd

Hayward, CA 94545

Setup Options

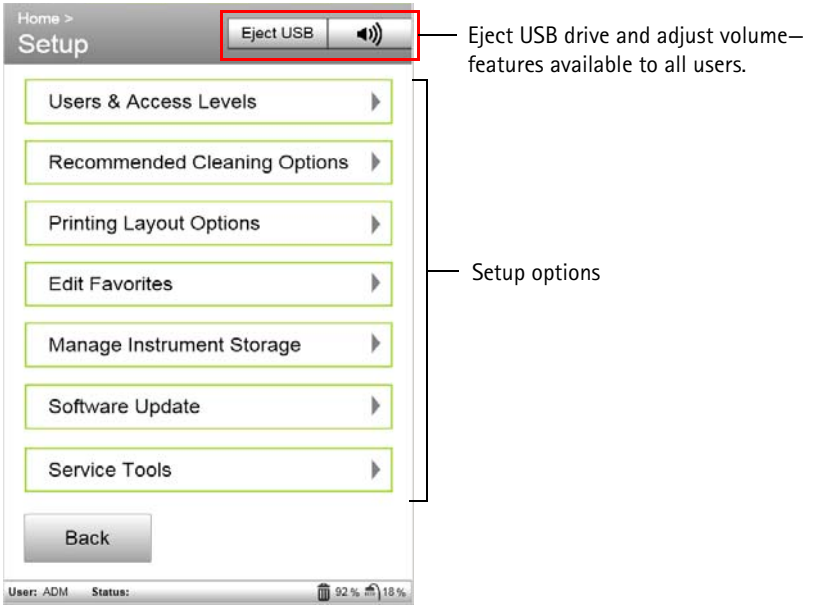
Setup contains the tools necessary to set various system features, such as adding new users and setting their access levels, selecting how often users should clean during an assay, and choosing printer and file storage options.

The Setup Screen

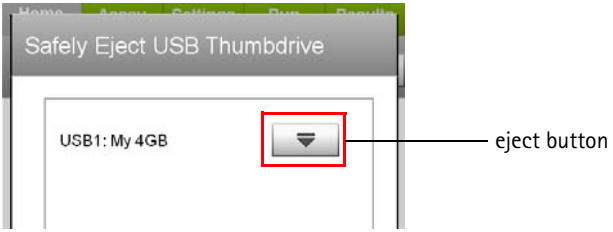
1 Select **Setup** from the main menu.

2 Select the option you wish to perform.

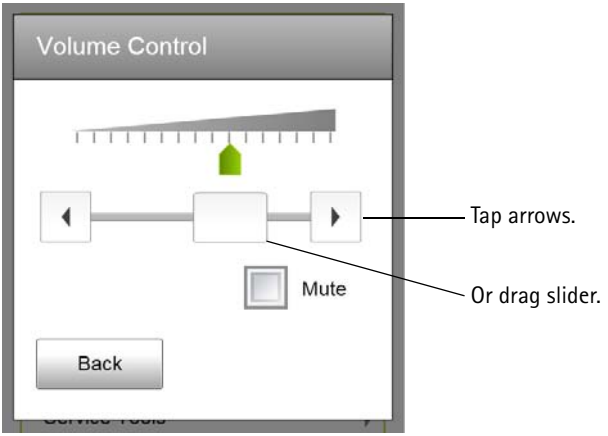
- **NOTE:** Depending on your user access level, some options may not be available to you. Refer to "Users & Access Levels" on page 79 for information on the options available for each user access level.



3 Select **Eject USB** in the title bar to safely eject a USB thumb drive. The USB drives currently connected to the instrument are listed. Select the eject button to the right of the drive you want to eject.



4 Select the volume control in the title bar to adjust the audio feedback on the instrument. Use the center slider or the arrows on either end of the slider to increase or decrease the volume. Select **Mute** if you wish to turn off the volume.



5 Refer to the following sections for more information on the Setup task you wish to perform.

Users & Access Levels

The Muse™ System supports two levels of access control—Administrator and Operator. The access level is assigned by the laboratory administrator when a new user is added to the system.

Administrators have privileges to all options within the Setup menu. Operators have the following privileges only:

- eject a USB drive
- adjust the volume
- edit their own user name and/or PIN
- select the default printer
- edit favorites (assay list that appears on the main menu)
- export their own data by user or date (however, cannot delete after exporting)
- export a Service Check file
- export the cleaning log

Tasks that operator-level users cannot perform are:

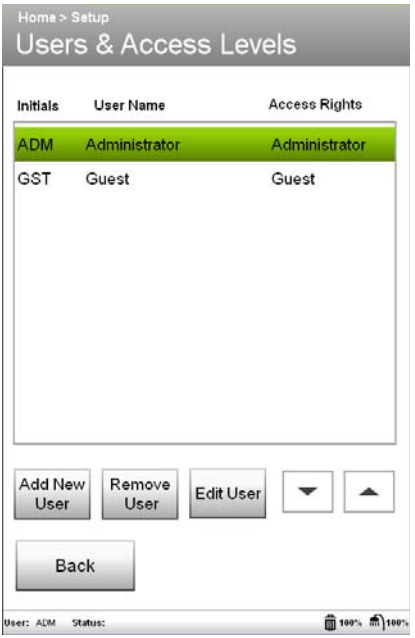
- change the cleaning options
- import or export all user data
- edit printing layout options
- update the software
- export the System Check log
- export data (or save instrument settings) to the Public folder

Accessing the Users & Access Levels Screen

1 Select **Setup** from the main menu.

2 Select **Users & Access Levels** from the Setup menu.

The Users & Access Levels screen appears. Administrators can add and remove users. Any user can edit their name and PIN.



3 Refer to the following sections for more information on adding, removing, and editing user information.

Adding a New User

Only administrators can add new users to the system. When you add the first new Administrator user, the default Administrator (ADM) will be deleted.

1 Select **Setup** from the main menu.

2 Select **Users & Access Levels** from the Setup menu.

3 Select **Add New User.**

■ **NOTE:** All fields are required.

4 Enter the user name using the keypad.

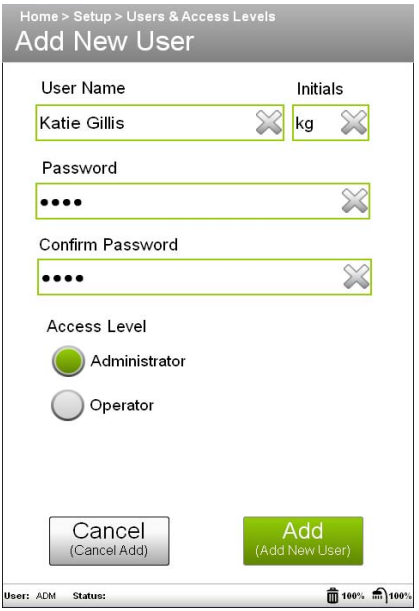
5 Enter up to 3 characters in the Initials field to identify the user. Select **Done**.

These initials will appear in the status bar when the user is logged on.

6 Enter up to 4 numeric characters for the password. Select **Done**. Confirm the password by reentering it. Select **Done**.

7 Select an access level—Administrator or Operator.

8 Select **Add**.



Removing a User

Only administrators can remove a user from the system. The default Administrator will be overwritten by the first Administrator-level user added to the system. The default Guest user cannot be removed.

■ **NOTE:** Removing a user does not remove the user's FCS data from the system.

1 Select **Setup** from the main menu.

2 Select **Users & Access Levels** from the Setup menu.

3 Select the user name from the list of users, then select **Remove User**.

4 Select **Yes** at the confirmation screen to remove the user.

Editing User Information

Any user can edit their user name and/or password. Only administrators can change a user's access level.

1 Select **Setup** from the main menu.

2 Select **Users & Access Levels** from the Setup menu.

3 Select the user name, then select **Edit User**.

4 Modify the information and select **Save**.

Recommended Quick Clean

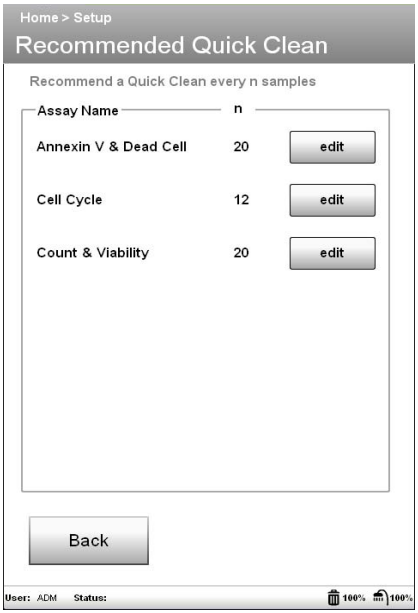
The Recommended Quick Clean feature allows your administrator to select how often a Quick Clean cycle should be performed during a run. When a user is running an assay a message appears prompting the user to perform a Quick Clean. Only administrators can modify the recommended Quick Cleans.

1 Select **Setup** from the main menu.

2 Select **Recommended Cleaning Options** from the Setup menu.

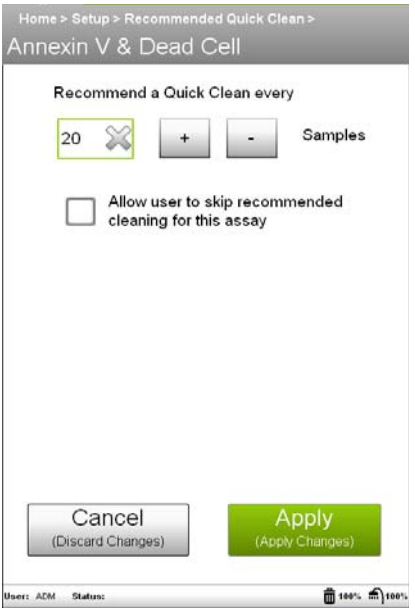
3 Select **edit** to change the current setting for a given assay.

The **n** represents the number of samples that can be run before the user will be prompted to perform a Quick Clean.



4 Use the + and – buttons to increase or decrease the number of samples between Quick Cleans. You can also select to allows users to skip the recommended cleaning for this assay. In this case, when the cleaning message appears during the assay, the user can select **Cancel** to skip the cleaning.

5 Select **Apply** to apply the changes.



Printing Layout Options

Any user can select the default printer. Only administrators can edit the layout options.

1 Select **Setup** from the main menu.

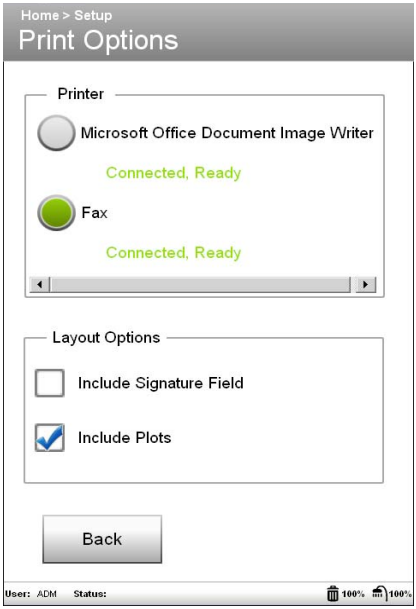
2 Select **Printing Layout Options** from the Setup menu.

3 Select the printer you wish to use as the default.

4 Select the layout options you wish to use.

- **Include Signature Field** adds a signature field to the printout.
- **Include Plots** includes plots on the printouts.

■ **NOTE:** Only administrators can edit the layout options.



5 Click **Back** to save changes.

Editing Favorites

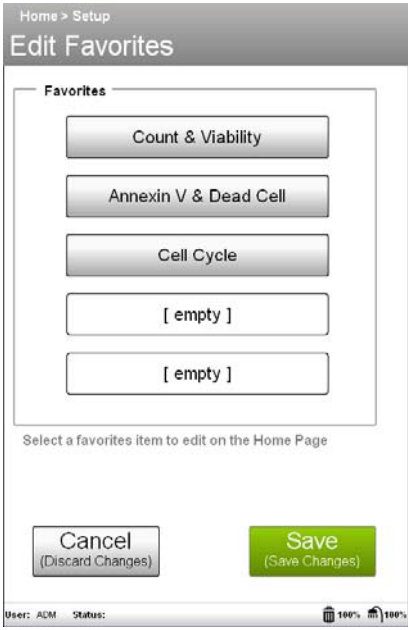
You can add assays and select the order in which they appear on the main menu using the Edit Favorites option. All user levels can add and organize assays in the Favorites list.

1 Select **Setup** from the main menu.

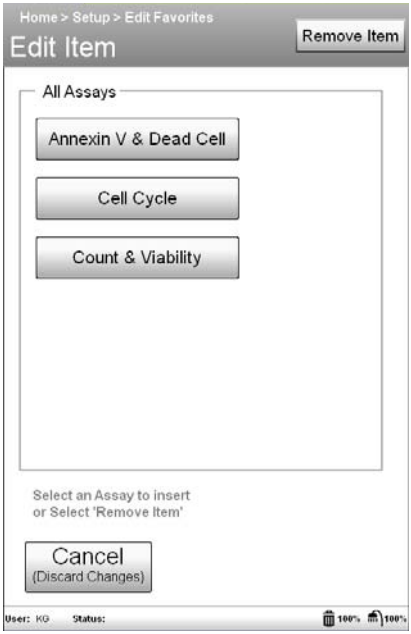
2 Select **Edit Favorites** from the Setup menu.

3 Select a slot, either with a current assay (to remove the assay) or an empty slot (to add an assay).

■ **NOTE:** If you want to change the order of the assays, first remove the assays then add them in the order you want them to appear.



4 Select the assay that you want to add (or replace) for the selected slot. Or, select **Remove Item** to remove the assay from the selected slot.



The Edit Favorites screen now appears with the selected assay in the slot (or the assay removed from the slot).

5 Select **Save.**

Managing Instrument Data Storage

The Manage Instrument Storage options allow you to import data to and export data from the system. Use the export features to back up or share data.

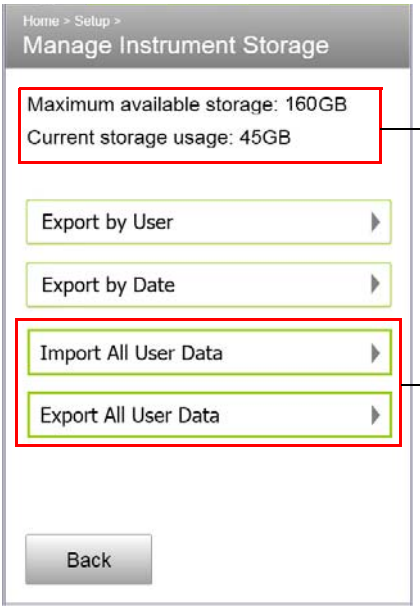
For a description of the folders created on the USB drive when you export data, see "Folder and File Structure" on page 25.

1 Select **Setup** from the main menu.

2 Select **Manage Instrument Storage** from the Setup menu.

3 Select the option you wish to perform.

Only administrators can import and export all user data. Operators can export their own data (by user and date).



4 Refer to the following sections for more information.

Exporting by User

You can export data by the user. Operator-level users can only export their own user data.

1 Select **Setup** from the main menu.

2 Select **Manage Instrument Storage** from the Setup menu.

3 Select **Export by User** to export data for a particular user.

4 Select the USB drive from the Export Location field.

5 Select the user whose data you wish to export from the Users list.

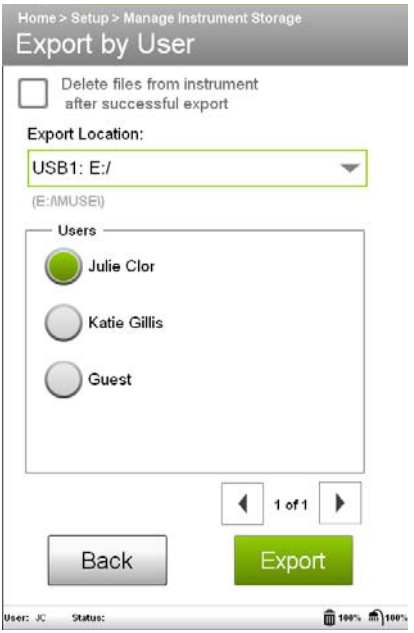
Use the arrow buttons in the bottom-right corner to scroll through the pages of users.

6 If you wish to delete the data once it has been successfully exported, select **Delete files from instrument after successful export**.

■ **NOTE:** Only administrators can delete data after exporting.

7 Select **Export**.

If you selected to delete the data after exporting, a dialog appears requesting confirmation. Select **Yes**. Otherwise, a message appears indicating the export status. Select **Close**.



Exporting by Date

You can export data from the last 30, 60, or 90 days or older. Operator-level users can only export their own user data.

1 Select **Setup** from the main menu.

2 Select **Manage Instrument Storage** from the Setup menu.

3 Select **Export by Date** to export data for a particular time frame.

4 Select the USB drive from the Export Location field.

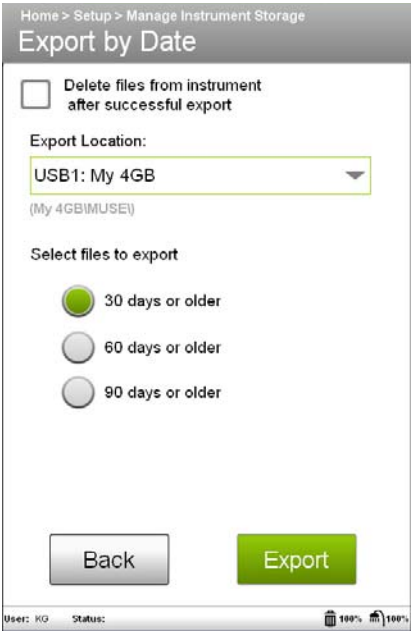
5 Select the files to export—30, 60, or 90 days or older.

6 If you wish to delete the data once it has been successfully exported, select **Delete files from instrument after successful export**.

■ **NOTE:** Only administrators can delete data after exporting.

7 Select **Export**.

If you selected to delete the data after exporting, a dialog appears requesting confirmation. Select **Yes**. Otherwise, a message appears indicating the export status. Select **Close**.



Importing All User Data

If you exported all user data, for example, if you sent your system out for depot service, you may wish to import the data back to the system. Only administrators can import all user data.

▲ **WARNING:** Importing all user data will overwrite any data that you currently have on your system.

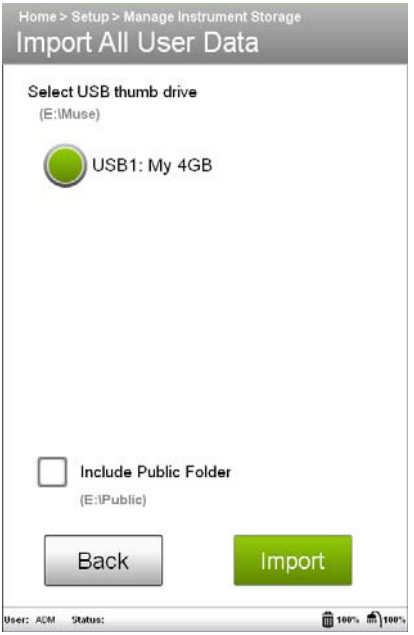
1 Select **Setup** from the main menu.

2 Select **Manage Instrument Storage** from the Setup menu.

3 Select **Import All User Data**.

4 Select the USB drive where the data is stored. If you wish to also import the data in the Public folder, select **Include Public Folder**.

5 Select **Import**.



6 The system automatically searches the drive for the user data. A message appears indicating that any data currently on the system will

be overwritten. Select **YES** to proceed, or **Cancel** to cancel the importing.

7 Select **Close** to the message that data was successfully imported.

Exporting All User Data

Use the Export All User Data feature to back up all data files. Only administrators can export all user data.

1 Select **Setup** from the main menu.

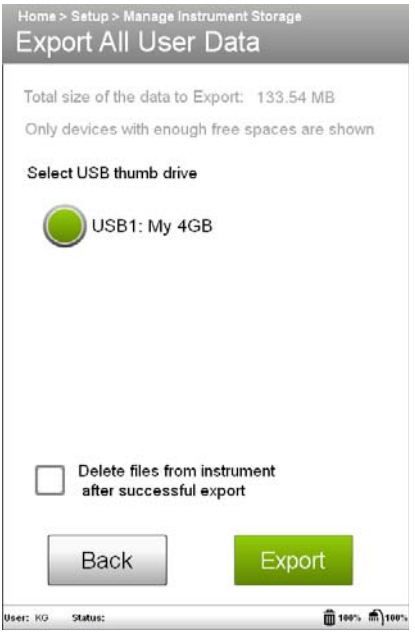
2 Select **Manage Instrument Storage** from the Setup menu.

3 Select **Export All User Data**.

4 (Optional) If you wish to delete the data from the system after it's exported, select **Delete files from the instrument after successful export**.

5 The system displays the drive space needed for the export and displays only the USB drives with the available space. Select the USB drive and then select **Export**.

■ **NOTE:** This could take several minutes depending on how much data you are exporting.



6 Select **Close** to the message that data was successfully exported.

Software Update

Use Software Update to load the latest version of Muse™ software on your instrument.

1 Download the software update from www.millipore.com onto a USB thumb drive.

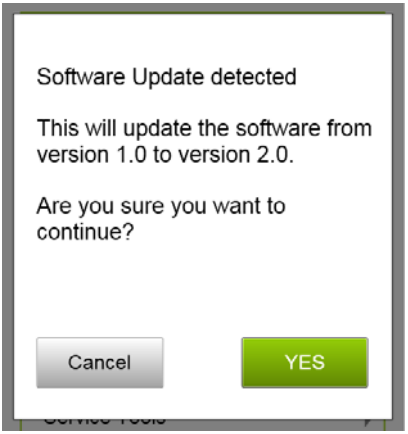
2 Install the USB drive into an available port on the instrument.

3 Select **Setup** from the main menu.

4 Select **Software Update** from the Setup menu.

The system automatically scans the available USB drives for a software update file. If an update is found, a confirmation message appears.

5 Select **YES** to install the software update.



Example

The new software will install and the system will automatically reboot in the new version. A message appears that the software successfully installed. The Select User screen appears.

Service Tools

Service Tools allows a qualified service technician to connect a laptop and run a service application for troubleshooting purposes.

Troubleshooting

This section lists possible problems you might encounter or messages you might see during operation. If you have a problem that you cannot resolve by using the troubleshooting section, call technical support, toll-free in the USA and Canada at 1 (800) MILLIPORE (645-5476) or visit www.millipore.com/techservice.

For assay specific troubleshooting, refer to the assay-specific kit user's guide at www.millipore.com/muse.

Problems

Problem	Recommended Solution
During startup, computer freezes on particular screens.	Cycle power by pressing the power button located below the touch screen.
Message: *Instrument could not be detected.*	Cycle power. If message appears after rebooting, contact Technical Support.
Instrument settings could not be retrieved	Ensure settings are located in the correct user folder. If settings are to shared among user, ensure settings files are saved to the Public folder.
Forgot user PIN	See "Forgetting Your User PIN" on page 39, or contact Tech Support.
Continued System Check failures after Complete System Clean.	Ensure the cleaning solution bottle is full of ICF and all connections are finger tight.
Loader arm is stuck in the up position. Eject button on screen does not lower it.	Use the loader arm release lever in the flow cell hatch. See "Loading and Unloading a Tube" on page 16.
Loader arm is not dropping/ loading.	Ensure the loader arm release lever in the flow cell access door is in the proper position (see page 16).

Problem	Recommended Solution
System Check failures: one ore more Particles/mL results fall outside the acceptance range (appears in red).	1.Ensure the correct Bead Lot # and Check Code are entered. Refer to the System Check Beads vial label and information card for values. 2.Run Quick Clean, then rerun the System Check procedure. If results still fail, run the Complete System Clean procedure. 3.Prepare a fresh bead sample and rerun the System Check procedure. 4.Rerun the Complete System Clean procedure. Ensure that the cleaning solution vial has sufficient fluid and that there are no kinks in the tubing from the flow cell or the cleaning solution vial.
Data sets saved by the default Administrator user are no longer accessible after a new Administrator user is added to the system.	The default Administrator is overwritten when a new Administrator user is added to the system. Add another administrator-level user with the name Administrator.
Sample acquisition is taking longer than expected or progress bar stops during acquisition.	Ensure that the System Check procedure was run and passed. If the progress bar stops during acquisition, the fluid system may be clogged. Run Quick Clean.
Instrument clogging; too many cells	Run Quick Clean to clean out the capillary. Resume running sample.
Lower Cell Concentration warning during acquisition	The sample concentration may be too low. The assay instructions are optimized to give you cell concentration between 100 and 500 cells/µL for accurate results. Repeat sample preparation with a lower dilution factor to allow for adequate cell numbers.
High Cell Concentration warning during acquisition	If the concentration of the stained cell sample for acquisition is high (>500 cells/µL), the accuracy of the data will most likely be compromised. Repeat sample preparation with a higher dilution factor to allow for adequate cell numbers.

Problem	Recommended Solution
Background staining and/or non-specific staining of cells	The cells may be damaged, as dead cells tend to aggregate and non-specifically bind fluorescent reagent. Avoid damaging the cells when handling and processing them in culture.
Low level of staining	Although the assay procedure was optimized for multiple cell types, every cell line behaves differently. A low signal may indicate that the cell concentration may be too high for the amount of reagent use. Restain cells at a lower concentration. Ensure proper controls are used.
Variability in day-to-day experiments	• If the results are inconsistent, check that the samples were well mixed prior to acquisition. Cells may quickly settle in your samples and your results will be inaccurate unless the cells are mixed just prior to acquisition. • Monitor experimental cell cultures to ensure that cell viability and cell numbers being analyzed are consistent. Any drop in cell numbers or viability can influence experimental results. • If there appears to be day-to-day variation of the staining pattern, ensure the Muse™ Cell Analyzer is working properly. Run the Muse™ System Check procedure to verify proper instrument function and accuracy. • Always monitor threshold settings, especially if using different cell types, to ensure cell events are not excluded.

Glossary

acquisition	The electronic and software function of collecting various types of information from a cell sample.
analysis	The software function of numerically and graphically manipulating data to identify and separate cell populations for the purpose of calculating relevant statistical information.
coefficient of variation (%CV)	The ratio of the standard deviation to the mean, expressed as a percent. It is calculated using the formula: $\%CV = \frac{SD}{\overline{X}} \times 100$
data set	A series of samples included within one file for a selected assay. An FCS file and a spreadsheet file are saved for each data set.
detector	A device used to measure light intensity. The fluorescence detectors are photodiodes (yellow and red). The FSC detector that measures cell size is also a photodiode. Both output a current that is proportional to the intensity of incident light.
dot plot	A graphical representation of two-parameter data. Each axis of the plot displays values for one parameter. A dot represents the values for a cell or particle.
FCS file	Flow Cytometry Standard file. A data file containing the results for an individual sample as well as all acquisition information at the time of data collection. FCS files are defined by the Data Files Standards Committee of the Society for Analytical Cytology. Cytometry. 1990;11:323–332.

flow cell	An optical assembly within the Muse™ Cell Analyzer. The flow cell consists of a metal shuttle holding a glass capillary with a tiny chamber where the laser beam illuminates the sample stream and cellular measurements occur.
fluorescence	The phenomenon of light emission that occurs when a fluorochrome's excited electrons drop to a lower energy level.
fluorochrome	A fluorescent dye used as a detection reagent in cell analysis applications. A molecule capable of absorbing light at a certain wavelength, then emitting light at a longer wavelength (fluorescence) as it releases energy.
gate	A graphical boundary that defines a subset of data. Gates may be set on a single-parameter histogram or a two-parameter dot plot.
histogram	A graphical representation of single-parameter data. The horizontal axis of the graph represents the increasing signal intensity of the parameter and the vertical axis represents the number of events (cells).
laser	Light amplification by stimulated emission of radiation. A light source that is highly directional, monochromatic, coherent, and bright. The emitted light is in one or more narrow spectral bands, and is concentrated in an intense, narrow beam.
marker	A boundary or set of boundaries used to segregate data into subsets for statistical analysis. Set a marker on a histogram to obtain statistics on a certain region. Set quadrant markers on a dot plot to obtain statistics on data within four quadrants.
mean fluorescence	The average of the fluorescence intensities of each event acquired within a given set of events.

median	The axis value for the event that falls in the middle of the distribution.
parameter	A specific cell property that is measured as the cell passes in front of the laser beam. Each parameter is the output from a photomultiplier (which measures fluorescence) or a photodiode (which measures forward scatter).
population	A group of cells that express similar values within one or more parameters. For example, cells that are positive for a particular antibody appear in the same location within a histogram or dot plot.
red detector	A device used for measuring light intensity. The red channel on the Muse™ Cell Analyzer can measure fluorescent light from dyes that emit signals similar to PE-Cy5.
threshold	The minimum level of discrimination to electronically eliminate unwanted signal. A threshold setting allows you to specify events you wish to acquire based on signal intensity of the event. Anything below the threshold in not acquired.
yellow detector	A device used for measuring light intensity. The yellow channel on the Muse™ Cell Analyzer can measure fluorescent light from dyes that emit signals similar to PE.

Ordering Information

For ordering information contact the nearest EMD Millipore office by calling 1 800 645-5476 or visiting us on our website at www.millipore.com/offices.

EMD Millipore and its distribution network will provide Muse® products to all sectors of life science research in certain countries outside North America and Europe.

Part	Catalog Number
Muse™ Cell Analyzer	0500-3115
cleaning solution bottle and corresponding fluid tubing	0110-7245
waste bottle and corresponding fluid tubing	0110-7250
flow cell assembly	0500-3120
flow cell cleaning tool	6000-2820
1.5-mL tubes, Eppendorf (quantity 500)	1000-0785
Muse™ Resource flash drive (software and user's guide)	0110-7880
Muse™ Cell Analyzer User's Guide	0110-7895

For research use only; not for use in diagnostic procedures.

Reagents for Muse™ System	Catalog Number
Muse™ System Check Kit (100 tests)	MCH100101
Muse™ Count & Viability Kit (100 tests)	MCH100102
Muse™ Count & Viability Kit (600 tests)	MCH600103
Muse™ Count & Viability Kit (200X)	MCH100104
Muse™ Annexin V & Dead Cell Kit (100 tests)	MCH100105
Muse™ Cell Cycle Kit (100 tests)	MCH100106
Muse™ Cell Dispersal Reagent (100 tests)	MCH100107

Specifications

Muse™ System

Physical Characteristics

instrument weight:	13.1 lb (5.94 kg)
instrument size	
height:	8.69 in (22.07 cm)
width:	8.12 in (20.62 cm)
depth:	11.11 in (28.22 cm)
connectors:	power – Kycon KPJX-PM back panel for motherboard – Intel D525MW five USB connectors—one on front and four on back of instrument

Operating Environment

temperature:	16–35°C (60–95°F)
external power supply (input voltage range):	100–240 VAC, 50/60 Hz 80 W
main unit (input voltage):	15VDC, 5A
fuse rating:	auto-resettable

Optics

laser:	532-nm green laser
forward scatter detector:	photodiode
fluorescence detectors:	photodiodes (YLW 576/28, RED 680/30)

Signal Processing

parameter dynamic range:	4.0 decade
pulse processing:	digital signal processing
time:	every particle time stamped

Fluidics

flow cell dimension:	rectangular capillary (1.5 mm x 0.8 mm) with 100-µm round bore
pump:	positive displacement
sample flow rate:	7 µL/min to 36 µL/min
cleaning / waste bottles:	50-mL plastic (co-polyester) bottles with screw tops
waste generation:	<40 mL
dead volume:	50 µL
sample concentration:	final particle concentration of 1 x 10^4 to 5 x 10^5 particles/mL
sample requirement:	as few as 2,000 cells/test; typically 25,000-100,000 cells/test, depending on the assay

Data Management

computer:	embedded Intel® ATOM-based computer, 160 GB data storage
data file structure:	output data file formats: • binary data storage in Flow Cytometry Standard (FCS) 3.0 format • spreadsheet results file in comma-separated value (CSV) format

Performance

counting accuracy:	±10%
counting precision:	≤10% CV

Approved Printers

Following is a list of supported printers:

- HP Officejet 100 Mobile Printer
- HP Officejet 6000 Wireless Printer
- HP Officejet Pro 8000 Printer Series – A809

Compliance

The Muse™ System contains a Class IIIb laser operating at 532 nm.

This product complies with:

- 21 CFR 1040.10 and 1040.11 except for deviations pursuant to Laser Notice No. 50, dated June 24, 2007
- Class I limits for exposure to laser radiation set by the Center for Devices and Radiologic Health (CDRH)

Symbols

Symbol	Meaning
	Attention, consult accompanying documents.
	Affixed in accordance with European Council Directive 73/23/EEC
	Danger, laser radiation
	In accordance with Canadian Standards Association
	Separate collection of waste at end of life as required by European Directives. Dispose of in accordance with the applicable country regulation.
	Dangerous voltage

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Index

T

U

V

W

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