VERSION 1

DEC 20, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.4r3l2215ql1y/v1

Protocol Citation: ronan.ocualain 2023. Automated 96 well plate based protein quantitation using a Beckman Biomek™ NxP workstation and a Pierce™ 660nm Protein Assay Kit. **protocols.io** https://dx.doi.org/10.17504/protocols.io.4r3l2215ql1y/v1

Protocol status: Working

Created: Dec 12, 2023

Automated 96 well plate based protein quantitation using a Beckman Biomek™ NxP workstation and a Pierce™ 660nm Protein Assay Kit V.1

In 3 collections

ronan.ocualain[1]

[1]University of Manchester

protocols.io Ambassadors | BioMS CRF, UoM | 1 more workspace

ronan.ocualain

ABSTRACT

These include

- sample lysis,
- protein extraction,
- solubilisation,
- estimation,
- reduction and alkylation,
- normalisation,
- clean-up,
- enzymatic digestion,
- and desalting.

Adapting these steps onto an automated workstation can increase efficiency, throughput, and reduce coefficients of variance (%CV) thereby providing reliable reproducible data for statistical comparisons.

This protocol is part of a modular collection for the processing of biological samples for proteomics.

Last Modified: Dec 20, 2023

PROTOCOL integer ID: 92191

Keywords: biochemistry, liquid chromatography - mass spectrometry (LC-MS), automation, protein sample preparation, proteomics, high-throughput, reproducibility, Beckman, Biomek, modular, Pierce, 660nm, Thermo, protein quantitation, protein estimation, normalisation, quantitation

GUIDELINES

A Beckman Biomek NxP with Span-8 pod and associated software is used in this method.
Of course, alternative liquid handlers can be used with appropriate method development.

The Biomek is a versatile liquid handler, but this means that alternative deck orientations and system components are possible. You may need to modify the method file for your specific Biomek liquid handler system.

MATERIALS

Equipment

	NAME
Beckman Coulter	BRAND
Biomek NXp	SKU

Equipment

96-well microplate	NAME
Pierce	BRAND
15041	SKU

Equipment

8 AFA-tube TPX strip holder	NAME
holder	TYPE
Covaris	BRAND
500685	SKU

Equipment

8 AFA-Tube TPX strip	NAME
Ultrasonication strip	TYPE
Covaris	BRAND
520292	SKU

Equipment

Full reservoir	NAME
reservoir	TYPE
Beckman	BRAND
372784	SKU

Equipment	
Biomek Tips P20 Sterile	NAME
tips	TYPE
Beckman	BRAND
717255	SKU

Equipment	
Biomek Tips P250 Non-Sterile	NAME
tips	TYPE
Beckman	BRAND
717251	SKU

Ionic Detergent Compatibility Reagent for Pierce™ 660nm Protein Assay Reagent **Thermo Fisher Catalog #22663**

Pierce™ 660nm Protein Assay Kit **Thermo Fisher Catalog #22662**

SAFETY WARNINGS

Wear PPE when operating.
Prepare solvents in a fume hood.
Store organic solvents in a flammable storage cabinet when not in use.
Discard used solvents and buffers in appropriate waste containers

BEFORE START INSTRUCTIONS

While this step is part of a modular format in the automated process of sample preparation for proteomics, it may also be performed in a stand-alone capacity. In the modular workflow it is usually performed after ultrasonication and reduction and alkylation of lysates.

In this method, the sample concentrations are assayed from either the Covaris LE220+ and either 96 AFA-Tube TPX plate (PN 520291), or 8 AFA -Tube TPX strip (PN 520292).

Other microplates for sample estimation may be used - plate information and location must be updated in the **Deck layout** and **Instrument setup** part of the method.

The method may be accessed here:

ProteinQuantitation660nmAssayV01.bmf297KB

Deck layout

1 To a Beckman full reservoir, add 50 mL of Pierce 660nm protein assay reagent with 1 g of ionic detergent compatibility reagent (IDCR). This can be prepared by adding the 1g from a packet of IDCR to a 50 mL falcon tube, and adding 50 mL of Pierce 660nm assay reagent, with mixing by inverting. Place the reservoir at P4 of the deck, and label it **Assayreagent**.

2 To the deck of the Biomek NxP, add a box of p250 tips to P8, and two boxes of p20 tips to P2, and P6 respectively.

3 Add the sample plate to be measured in P1 of the deck.

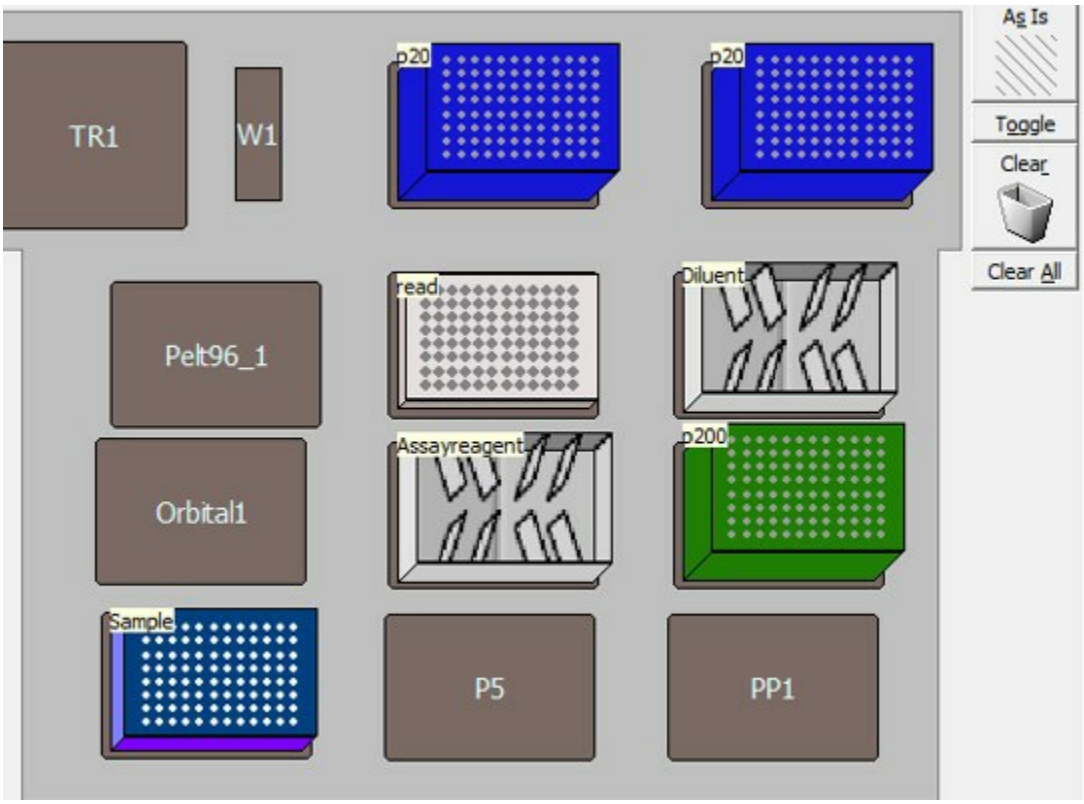
Note

The sample plate in this case is either a Covaris 96 AFA-Tube TPX plate (PN 520291), or 8 AFA -Tube TPX strip (PN 520292).
The correct plate details for your samples may be modified here and in **Instrument setup**.

4 To a Beckman full reservoir, add 50 mL of deionised water, and place in P7 of the deck, label this **diluent**.

5 Add a Pierce microplate to P3, and label it **read**.

6 The deck should look like the following



Deck layout

Setting up the deck

7 Double click the software icon.

8 Under the Method tab, select home all axes to orient and prepare the automated liquid handler.

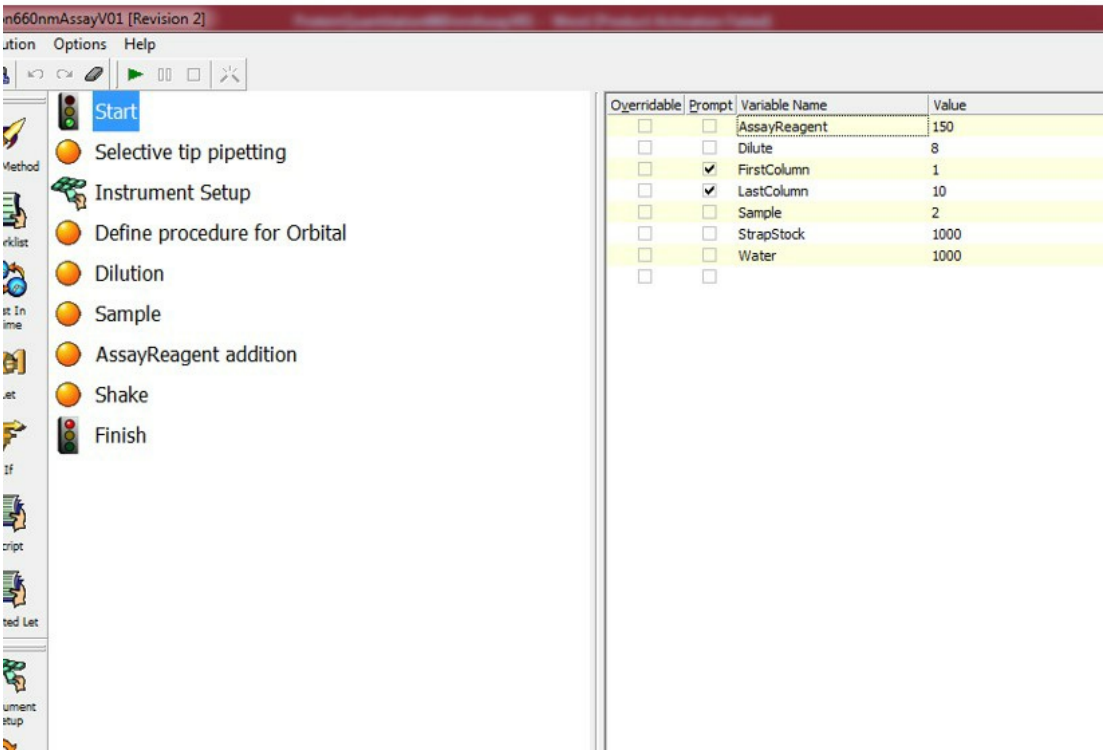
9 Under File, select Open/Method. Select the ProteinQuantitation660nmAssayV01 method.

10 To the deck of the NxP, add two p20 tip boxes, and 1 p250 tip box.
The **Sample** will be in **P1** position, while the **AssayReagent** is at **P4**. The **diluent** will be at **P7**.

11 Add a **Read** plate to **P3** (A Pierce 96 well microplate #15041 in this case).
It is at this step that the calibrants for the generation of the standard curve be added, in columns 11 and 12. I recommended using Thermo Scientific™ Pierce™ Bovine Serum Albumin Standard Pre-Diluted Set (23208). These are a set of 7 protein standards ranging from [M] 2 µg/µL to [M] 0.125 µg/µL .
The last available pair of wells can be used for the diluent blank.

Operating procedure

12 The default liquid sample volume for this method is 120uL.
Volumes may be changed in plate properties, and the dilution ratio of the samples to be measured may be adjusted in the **Start** tab of the program

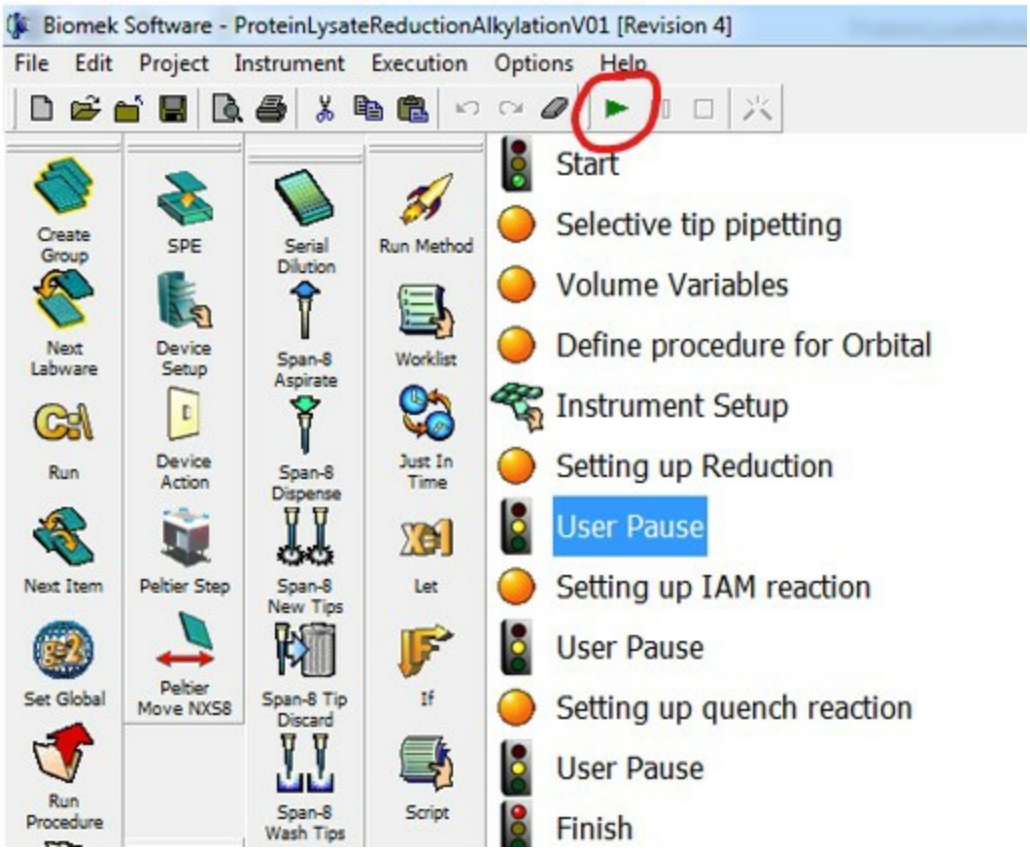


Start Menu

The dilution in this assay of sample is 1:5, where 2 µL of sample is added to 8 µL of diluent.

Running the assay

13 Start the method by clicking the green Run icon.



Start

14 You will be prompted by the software to enter the location of the first column to be processed.
The method is set up to transfer and dilute columns 1 to 10 of the sample plate to the read plate.
This is because columns 11 and 12 are used for generating the standard curve.
If your first samples are in column A1, enter **"1"**
You will then be prompted to enter the value of the last column to be processed. If you have a full plate of 80 samples, enter **"10"**. All 80 wells will be processed.

15 The software will ask you to check that the deck layout matches that of the program. Once you are satisfied that this is the case, click OK.

The instrument will now dilute, and add assay reagent to your diluted samples.

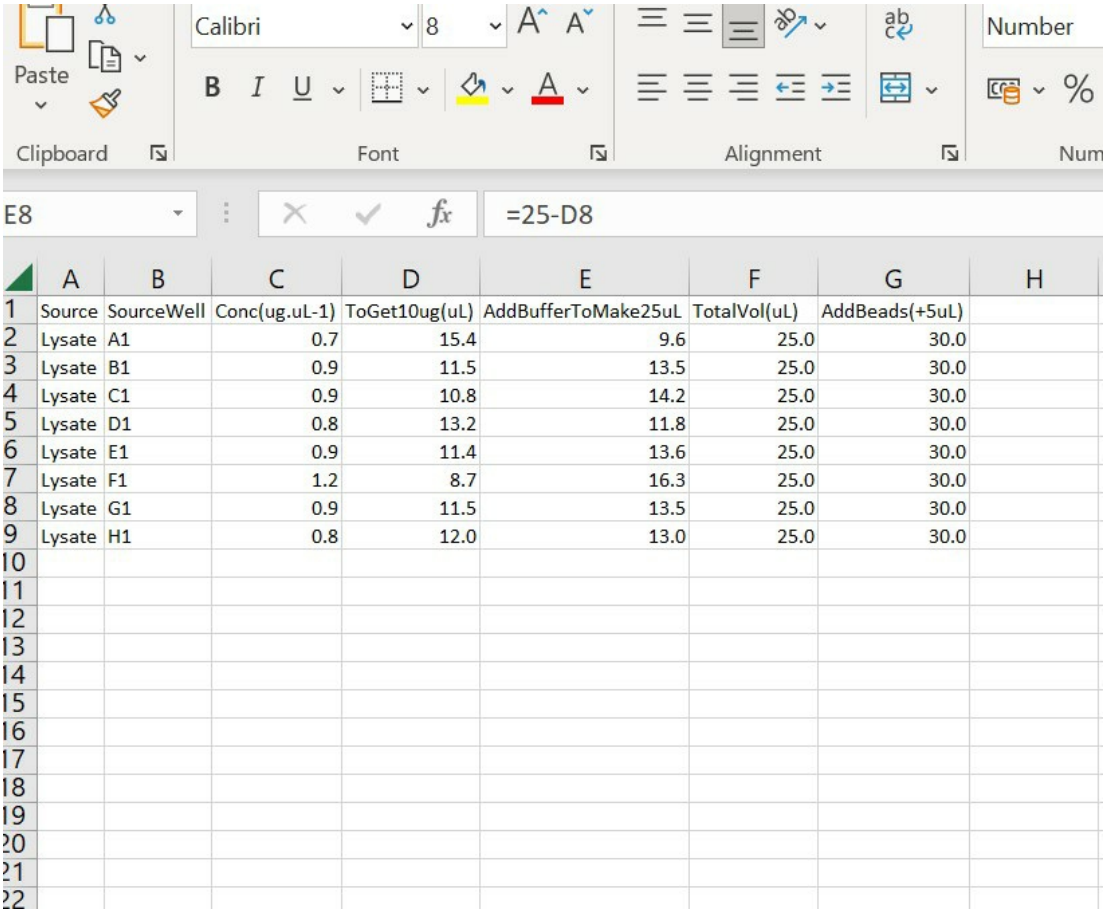
Measurement

16 Once shaking of the plate has completed, you may now measure the plate using endpoint absorbance at a wavelength of 660nm to measure the protein concentration of samples from columns 1 to columns 10. Columns 11 to 12 are used to set up the calibration curve.

17 The results may be exported to an .csv file, and transformed to provide volumes for normalisation of samples prior to processing, clean-up, and digestion.

Data transformation

18 Data from the 660nm assay may be edited as below:

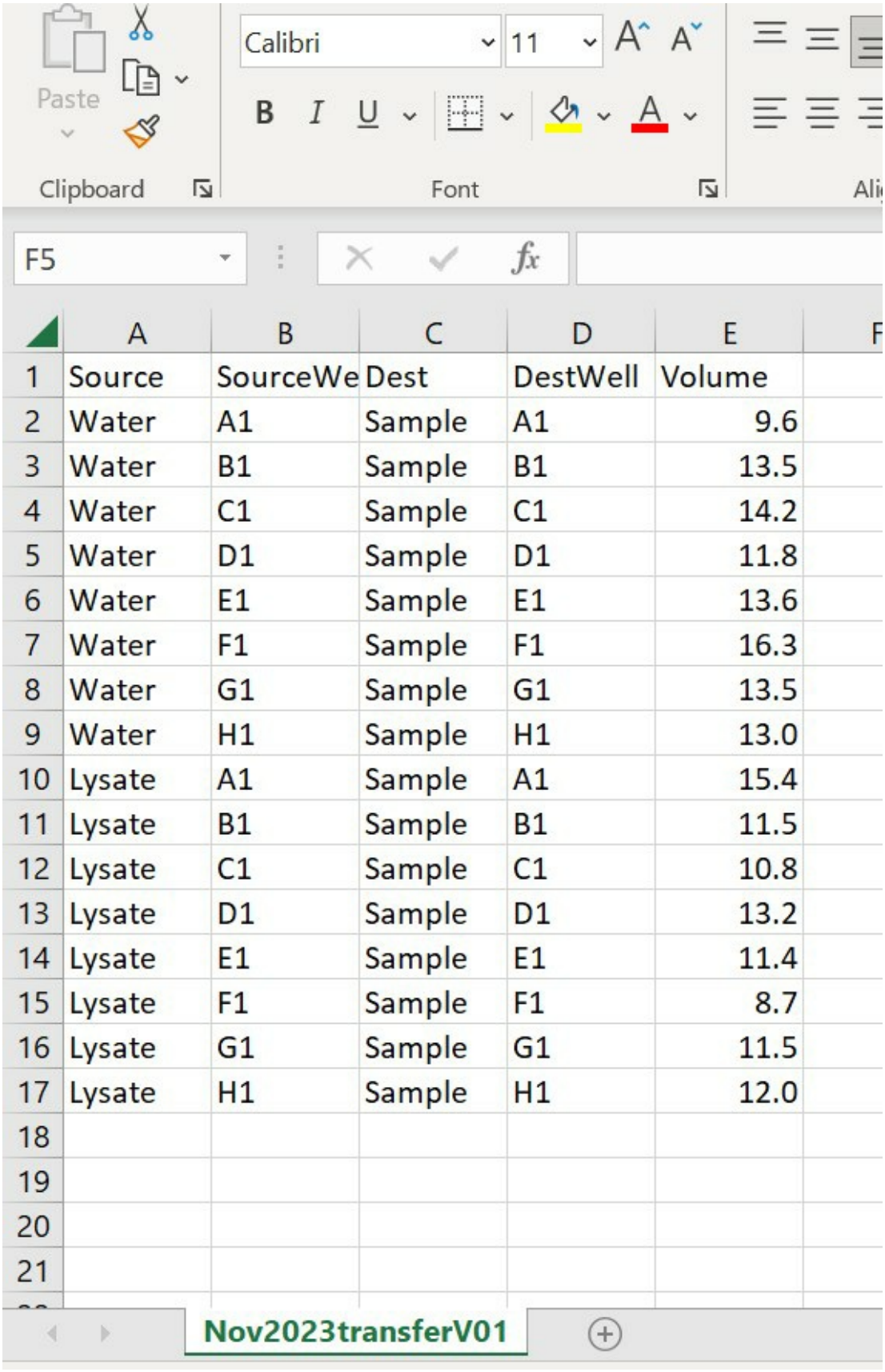


	A	B	C	D	E	F	G	H
1	Source	SourceWell	Conc(ug.uL-1)	ToGet10ug(uL)	AddBufferToMake25uL	TotalVol(uL)	AddBeads(+5uL)	
2	Lysate	A1	0.7	15.4	9.6	25.0	30.0	
3	Lysate	B1	0.9	11.5	13.5	25.0	30.0	
4	Lysate	C1	0.9	10.8	14.2	25.0	30.0	
5	Lysate	D1	0.8	13.2	11.8	25.0	30.0	
6	Lysate	E1	0.9	11.4	13.6	25.0	30.0	
7	Lysate	F1	1.2	8.7	16.3	25.0	30.0	
8	Lysate	G1	0.9	11.5	13.5	25.0	30.0	
9	Lysate	H1	0.8	12.0	13.0	25.0	30.0	
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
20								
21								
22								

Calculation of protein amounts

In the above example, individual protein lysates were measured in wells A1 to H1. From this, the volume of lysate needed to process 10 µg was calculated, along with the amount of HEPES buffer to give a final volume of 25 µL .

19 This data may then be used to make a .csv file as detailed below



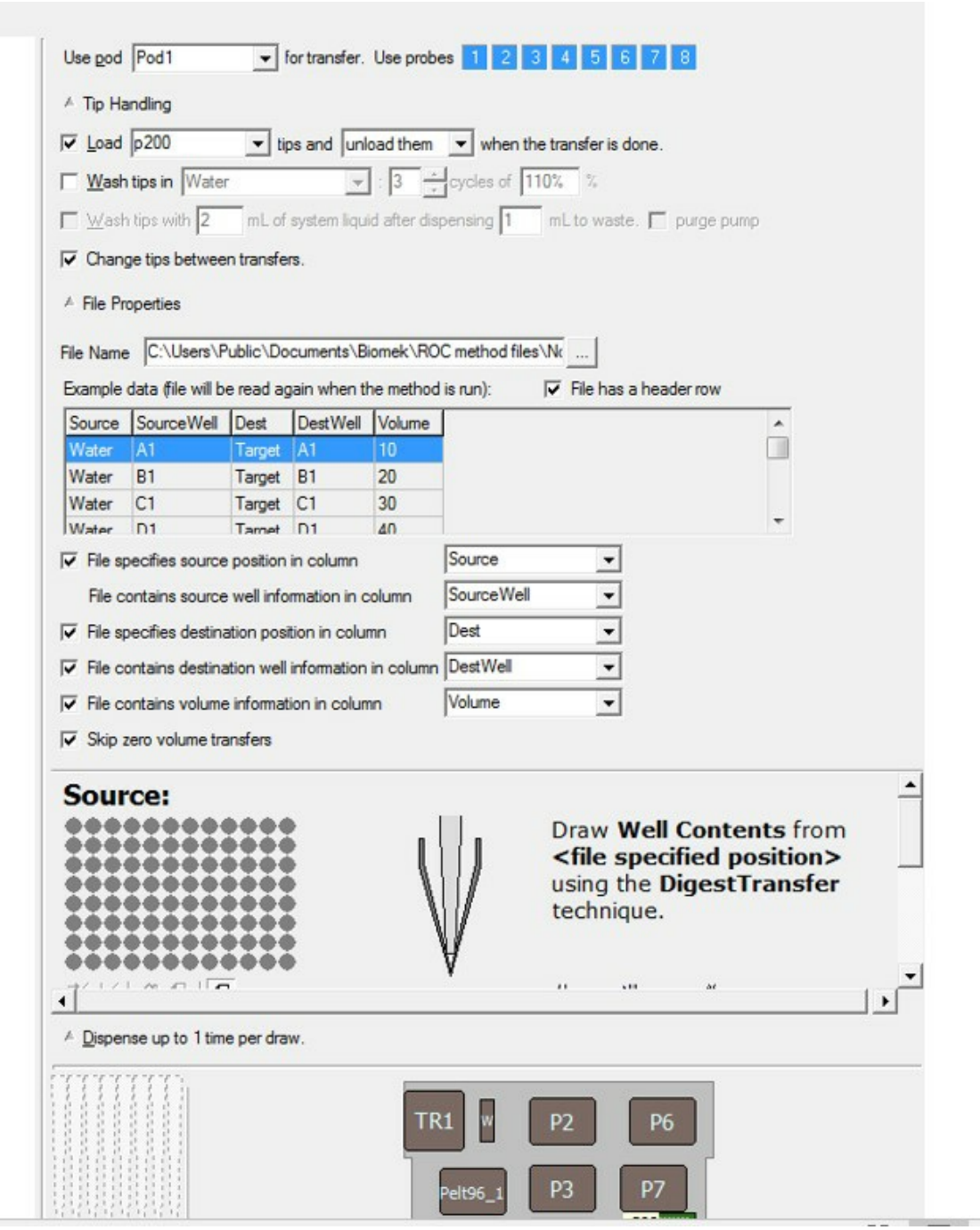
	A	B	C	D	E
1	Source	SourceWe	Dest	DestWell	Volume
2	Water	A1	Sample	A1	9.6
3	Water	B1	Sample	B1	13.5
4	Water	C1	Sample	C1	14.2
5	Water	D1	Sample	D1	11.8
6	Water	E1	Sample	E1	13.6
7	Water	F1	Sample	F1	16.3
8	Water	G1	Sample	G1	13.5
9	Water	H1	Sample	H1	13.0
10	Lysate	A1	Sample	A1	15.4
11	Lysate	B1	Sample	B1	11.5
12	Lysate	C1	Sample	C1	10.8
13	Lysate	D1	Sample	D1	13.2
14	Lysate	E1	Sample	E1	11.4
15	Lysate	F1	Sample	F1	8.7
16	Lysate	G1	Sample	G1	11.5
17	Lysate	H1	Sample	H1	12.0
18					
19					
20					
21					

Transfer .csv file

20 Once this is complete, modify the file

LysateTransferV01.bmf296KB

and add the .csv file to it as follows



transfer step

Upload the .csv file prepared earlier in the **file name** tab.
Note, it is important that the headers match, in this case, these are **Source, sourcewell, dest**, and **dest well**, along with **volume**.