

Total Lysate Prep and BCA Protein Assay	
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Total Lysate Prep and BCA Protein Assay

I. Purpose

To provide instruction of how to extract the total lysate from a frozen cell pellet and to measure the protein concentration in the total lysate using a BCA Protein Assay

II. Scope

All procedures are applicable to the BCGSC Library Core and Library Technology Development groups

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a QA associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Document Title	Document Number
Pierce® BCA Protein Assay Kit Instructions	23227

VI. Related Documents

Document Title	Document Number
96-well DNA Quantification using the dsDNA Quant-iT High Sensitivity Assay Kit and VICTOR ³ V	LIBPR.0108

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the material safety data sheet (MSDS) for additional information.

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VIII. Materials and Equipment

Name	Supplier	Number: #	Model or Catalogue #	
Fisherbrand Textured Nitrile gloves	Fisher Scientific	270-058-53		✓
Ice Chest	Igloo	PM PAL BLUE		✓
wet ice	In house	N/A	N/A	N/A
DNA away	Molecular Bioproducts	7010		✓
Anhydrous Ethyl Alcohol (100% Ethanol)	Commercial Alcohol	People Soft ID: 23878		✓
Large Kimwipes	Fisher Scientific	06-666-117		✓
Black ink permanent marker pen	VWR	52877-310		✓
Small Autoclave waste bags 10"X15"	Fisher Scientific	01-826-4		✓
Gilson P10 pipetman	Mandel	GF-44802		✓
Gilson P20 pipetman	Mandel	GF23600		✓
Gilson P200 pipetman	Mandel	GF-23601		✓
Gilson P1000 pipetman	Mandel	GF-23602		✓
Neptune barrier tips 10 µl	CLP	Bt10XL		✓
Neptune barrier tips 20 µl	CLP	Bt20		✓
Neptune barrier tips 200 µl	CLP	Bt200		✓
Neptune barrier tips 1000 µl	CLP	Bt1000		✓
RIPA buffer	Thermo Scientific	89900		✓
Halt Protease Inhibitor Cocktail (100X)	Thermo Scientific	78415		✓
Centrifuge, Eppendorf 5417R, refrigerated high-speed, 115V	Fisher Scientific	5417R	✓	
Pierce® BCA assay kit	Thermo Scientific	23227		✓
1.5 ml Eppendorf tube	Ambion	12400		✓
15ml Conical Tubes	VWR	CA21008-918		✓
5ml serological pipettes	Fisher Scientific	CS004487		✓
Room temperature Centrifuge, Large	Eppendorf	5810R	✓	
Costar® UV-Transparent Microplates	Costar	3635		✓
Victor ³ V Multilabel Counter	PerkinElmer	1420	✓	

IX. Procedure

Total Lysate Prep

1. Retrieval of reagents and equipment preparation in the ChIP Room

- 1.1. Put on a clean pair of gloves and lab coat.
- 1.2. Wipe down the work bench, small equipment, and ice bucket with DNA away and 80% Ethanol.
- 1.3. Change gloves and retrieve fresh ice.

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- 1.4. Retrieve RIPA Buffer (1X) and Halt Protease Inhibitor Cocktail EDTA free (100X) from the 4°C fridge and place on ice.
- 1.5. Fast cool the 4°C centrifuge.
- 1.6. Fill out and print off the worksheet in the Lysate Prep tab of the BCA Assay Template worksheet. The worksheet can be found at :\\Library Core\\Epigenomics\\BCA\\BCA_Assay_Template.
 - 1.6.1. Create a new folder in the :\\Library Core\\Epigenomics\\BCA\\ folder with the date of the experiment. E.g. 150106 for January 6th 2015.
 - 1.6.2. Click the "Lysate Prep" tab and print out the sheet.
 - 1.6.3. Click the "BSA Standards" tab and fill in the number of cell types in the cell highlighted in yellow. Print out this sheet.
 - 1.6.4. Click the "Plate Set-Up" tab and fill in the names of the cell types in the cells highlighted in yellow. Also fill in the Wells and Rows that will be used for the assay. Print out the prepared plate layout.
 - 1.6.5. Click "Save As..." and add the name of the cell types, the date and initials to the file name and save the file in the folder created in Step 1.6.1.

2. Preparing solutions and Retrieval of Total Lysate

- 2.1. Aliquot 3mL of RIPA buffer to a 15mL falcon tube, add 30µL of Halt Protease Inhibitor Cocktail EDTA free (PIC) to the RIPA Buffer.
- 2.2. Thaw a cell pellet. If the pellet contains 50 million cells or less, resuspend the cells in 170µL RIPA plus PIC. If the pellet contains more than 50 million cells, consult an APC for the appropriate volume of RIPA plus PIC.
- 2.3. Transfer the resuspended pellet into a 1.5mL microcentrifuge tube.
- 2.4. Incubate the tube on ice for 20 minutes.
- 2.5. Centrifuge at 14000g for 15 minutes at 4°C.
- 2.6. Transfer the supernatant to a 1.5mL tube.

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- 2.7. In a new 1.5mL tube, prepare a 1/5, 1/10, 1/20 dilution in duplicate of the total lysate for use in a BCA protein assay. The volume of each dilution should be 25µL.
- 2.8. Store the total lysate and the dilutions of the total lysate at 4°C if continuing on with a BCA protein assay the same day or following day. If not, store the total lysate at -20°C.

BCA Protein Assay

3. Preparation of Diluted Albumin (BSA) standards

- 3.1. In 9 labeled 1.5mL tubes, dilute the contents of one Albumin Standard (BSA) ampule with RIPA plus PIC. Prepare each protein standard using Table 1 as a guide. There is sufficient volume for three replications of each diluted standard.

Table 1. Dilution Scheme (Working Range = 20-2,000µg/mL)

<u>Vial</u>	<u>Volume of Diluent (RIPA plus PIC)</u>	<u>Volume and Source of BSA</u>	<u>Final BSA Concentration</u>
A	0	300 µL of Stock	2,000 µg/mL
B	125µL	375 µL of Stock	1,500 µg/mL
C	325µL	325 µL of Stock	1,000 µg/mL
D	175µL	175 µL of vial B dilution	750 µg/mL
E	325µL	325 µL of vial C dilution	500 µg/mL
F	325µL	325 µL of vial E dilution	250 µg/mL
G	325µL	325 µL of vial F dilution	125 µg/mL
H	400µL	100 µL of vial G dilution	25 µg/mL
I	400µL	0	0 µg/mL = Blank

- 3.2. Store the protein standards on ice until ready for use.

4. Preparation of the BCA Working Reagent (WR)

- 4.1. Use the following formula to determine the total amount of WR required for the assay:
 $(9 \text{ standards} + \# \text{ unknowns}) \times (\# \text{ replicates}) \times (200\mu\text{L of WR per sample}) = \text{Amount of WR}$

Example: 2 unknowns and 2 replicates of each sample:

$(9 \text{ standards} + 2 \text{ unknowns}) \times (2 \text{ replicates}) \times (200\mu\text{L of WR per sample}) = 4400\mu\text{L of WR}$

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4.2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part BCA Reagent B. For the above example, combine 5mL of Reagent A with 100µL of Reagent B.

5. Measurement of the HL60 total lysate and 1/10 diluted HL60 total lysate on the Victor³V

5.1. Set-up the plate using the well layout prepared in Step 1.6.4. (See Table 2 for an example well layout).

Table 2. Well Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Vial A	Vial B	Vial C	Vial D	Vial E	Vial F	Vial G	Vial H	Vial I	1/5 Dilution Cell Lysate	1/10 Dilution Cell Lysate	1/20 dilution Cell Lysate
B	Vial A	Vial B	Vial C	Vial D	Vial E	Vial F	Vial G	Vial H	Vial I	1/5 Dilution Cell Lysate	1/10 Dilution Cell Lysate	1/20 dilution Cell lysate

5.1.1. Pipette 200µL of the WR to each well and cover the plate with a plate seal.

5.1.2. Add 25µL of each protein standard, cell lysate and the diluted series of total lysate replicates into a microplate well.

5.1.3. Mix the plate thoroughly on a plate shaker for 30 seconds.

5.1.4. Spin down the plate in a room temperature centrifuge for 1 minute at 1000rpm.

5.1.5. Seal the plate and incubate at 37°C for 30 minutes.

5.1.6. Cool the plate to room temperature for 10 minutes.

5.1.7. Spin down the plate in a room temperature centrifuge for 1 minute at 1000rpm.

5.2. Set-up Victor³V software.

5.2.1. Sign into the Victor³V computer.

5.2.2. Start the Wallac 1420 Workstation on the desktop.

5.2.3. Select Tools from the toolbar and select Start Wizard. Click next.

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5.2.4. Select the BCA protocol and click next.

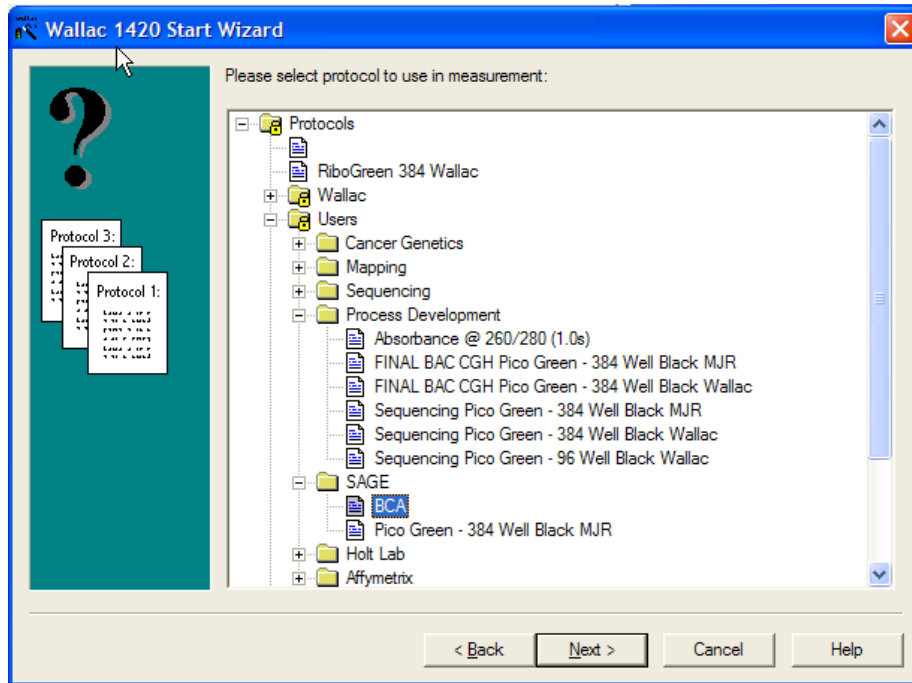


Figure 1. BCA Protocol

5.2.5. Select the wells that contain sample and click next.

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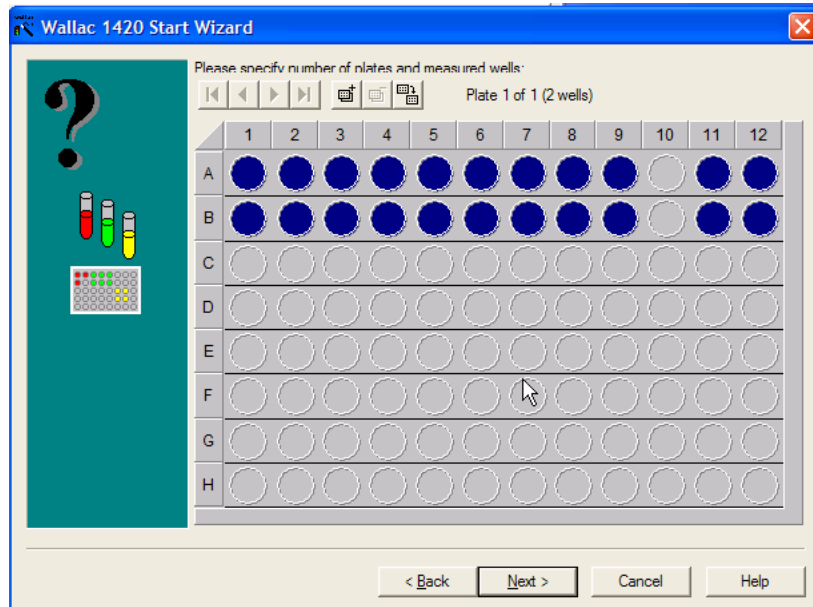


Figure 2. Well Selection

5.2.6. Insert Assay Name, Cell Type, # of cells and Experiment Date. Click next.

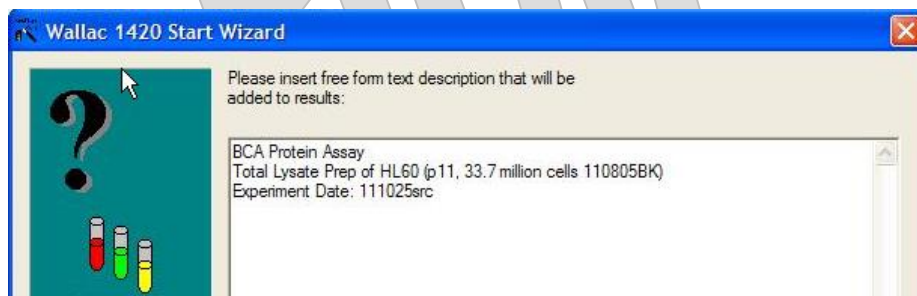


Figure 3. Insert Assay Name, Cell Type, # of cells and Experiment Date

5.2.7. Remove the cover seal off the plate and insert the plate into the instrument.

5.2.8. Click 'Finish' on the program to start the run.

5.2.9. After the run completes, select Tools in the toolbar and select Results of Latest Assay Run.

5.2.10. Export the results to an Excel file. Select File in the toolbar and select Export.

5.2.11. Save the file in the folder that was created in Step 1.6.1. E.g. :\\geneexplab\\Library Core\\Epigenomics\\BCA folder\\150106.

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6. Analysis of BCA Protein Results

6.1. Copy over the table in the “List ; Plate 1 -1” tab in the created file and copy it to the “Plate Set-Up” Tab in the edited BCA Template file created in Step 1.6. A standard curve will be automatically created in the “Results” tab of the BCA file. It plots the average absorbance value of each standard solution vs. the total amount of protein in each standard.

Table 3. Standard Dilution Setup

Standard Dilutions Setup						
Tube	Standard Vol	Source	Diluant Vol	ug/ml [Protein]	Total Protein ug	Absorbance
A	25	300uL stock	0	2000	50.00	1.199
B	25	375uL stock	125	1500	37.50	1.029
C	25	325uL stock	325	1000	25.00	0.755
D	25	175uL from B	175	750	18.75	0.612
E	25	325uL from C	325	500	12.50	0.493
F	25	325uL from E	325	250	6.25	0.324
G	25	325uL from F	325	125	3.13	0.236
H	25	100uL from G	400	25	0.63	0.159
I	25	0	400	0	0.00	0.142

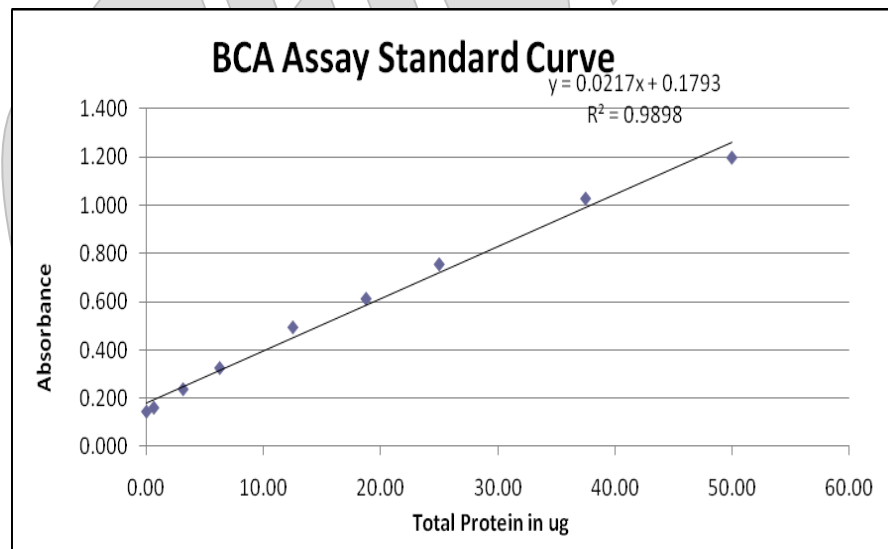


Figure 4. Standard Curve

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6.2. Using the standard curve, enter the values from the $y = nx + b$ equation into the highlighted yellow cells in the “Results” tab to determine the protein concentration of the cell lysate and the diluted series of cell lysate.

7. Aliquoting Lysate

7.1. For ease of use for future protocols, the lysate can be aliquoted into 200 μ L strip tubes, or individual PCR tubes.

- 7.1.1. Determine the volume of 25 μ g of lysate.
- 7.1.2. Aliquot out into strip tubes or individual PCR tubes.
- 7.1.3. Top up to 10 μ L with RIPA + PIC buffer, if needed.
- 7.1.4. Store in the -20 $^{\circ}$ C freezer.