*Non Controlled Version*

**Note: Controlled Versions of this document are subjected to change without notice*

# 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 Assy 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.

## 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.

1.4. Retrieve RIPA Buffer (1X) and Halt Protease Inhibitor Cocktail EDTA free (100X) from the 4ºC fridge and place on ice.

1.5. Fast cool the 4ºC 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 6$^{th}$ 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 4ºC.

2.6. Transfer the supernatant to a 1.5mL tube.

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 4ºC if continuing on with a BCA protein assay the same day or following day. If not, store the total lysate at -20ºC.

**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)**

| Vial | Volume of Diluent (RIPA plus PIC) | Volume and Source of BSA | Final BSA Concentration |
|---|---|---|---|
| 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 standards + # unknowns) x (# replicates) x (200µL of WR per sample) = Amount of WR

Example: 2 unknowns and 2 replicates of each sample:
(9 standards + 2 unknowns) x (2 replicates) x (200µL of WR per sample) = 4400µL of WR

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 Victor$^3$V

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 Celllysate |
| **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 37ºC 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 Victor$^3$V software.

5.2.1. Sign into the Victor$^3$V 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.

5.2.4. Select the BCA protocol and click next.

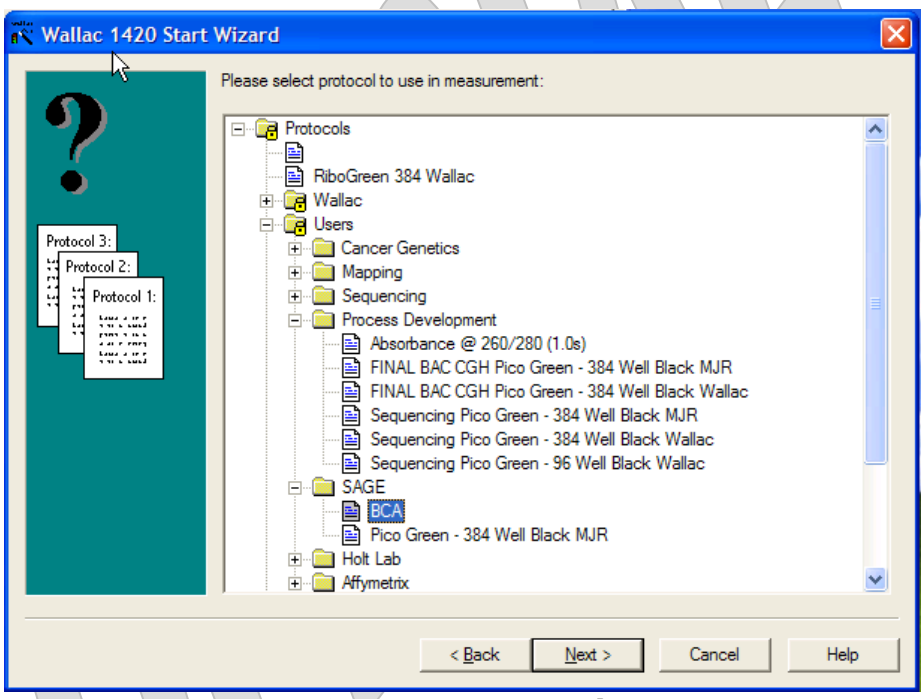


**Figure 1. BCA Protocol**

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

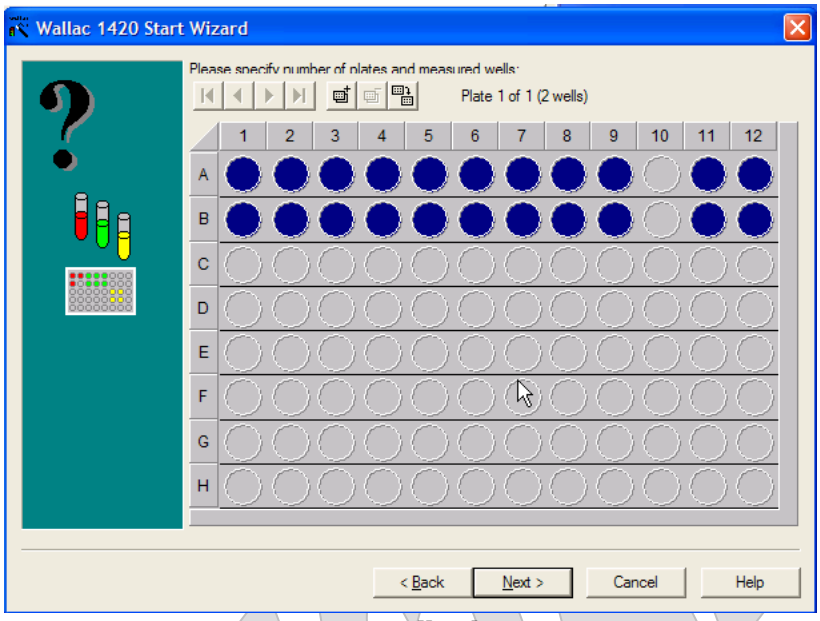


**Figure 2. Well Selection**

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

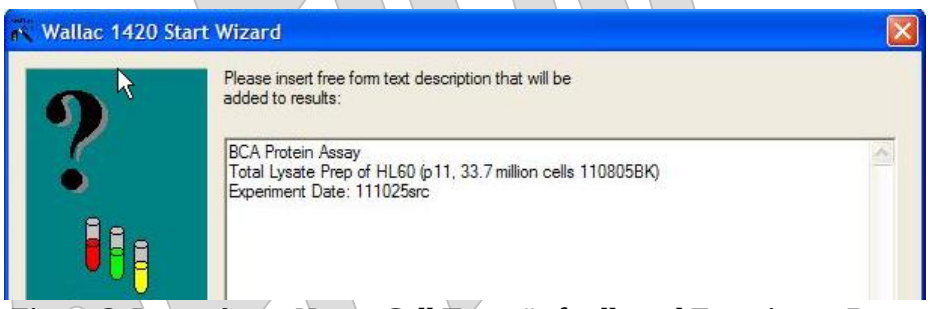


**Figure 3. Insert Assay Name, Cell Type, # of cells and Experiment Date**

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.

## 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 |

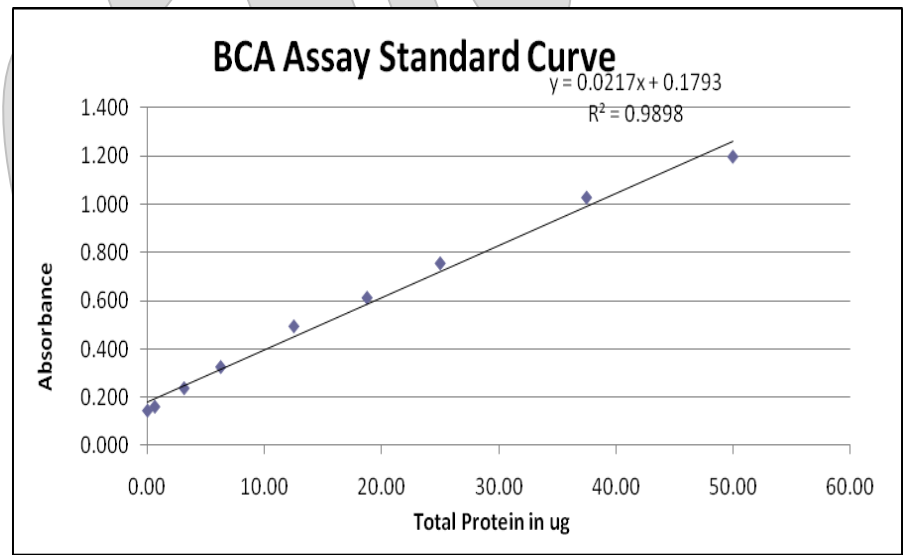


**Figure 4. Standard Curve**

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 200µL strip tubes, or individual PCR tubes.

7.1.1. Determine the volume of 25µg of lysate.
7.1.2. Aliquot out into strip tubes or individual PCR tubes.
7.1.3. Top up to 10uL with RIPA + PIC buffer, if needed.
7.1.4. Store in the -20°C freezer.