Protein Quantitation

1. Purpose

To measure the protein concentration of mouse hippocampal tissue extracts prepared as described in the protocol "Hippocampal Protein Extraction".

2. Procedure

This method is based on the Pierce Micro BCA Protein Assay kit (Materials) manufacturers instructions.

Prepare 100uL aliquots of the albumin 2mg/mL ampule for storage at -20 ^{o}C.

Prepare 2-fold dilution series of albumin in Milli-Q H_20 as follows;

- Take 100 uL 2 mg/mL albumin, add 5 mL Milli-Q H_20 to yield 40 ug/mL albumin. Mix.
- Take 2.5 mL 40ug/mL standard, add 2.5 mL Milli-Q H_2O to yield 20 ug/mL. Mix.
- Take 2.5 mL 20ug/mL standard, add 2.5 mL Milli-Q H_2O to yield 10 ug/mL. Mix.
- Take 2.5 mL 10ug/mL standard, add 2.5 mL Milli-Q H_2O to yield 5 ug/mL. Mix.
- Take 2.5 mL 5ug/mL standard, add 2.5 mL Milli-Q H_2O to yield 2.5 ug/mL. Mix.
- Prepare 0 mg/mL dilution with Milli-Q H_20.

Prepare duplicate 1 mL aliquots of each albumin standard (40, 20, 10, 5, 2.5 & 0 ug/mL) in 2 mL tubes. Spike 5uL of the relevant protein extraction buffer (i.e. DOC lysis buffer) into each tube containing a 1mL standard aliquot to correct for any detergent interference with the BCA assay.

Thaw an aliquot of protein extract to be quantitated on ice. Take 5 uL of extract and add Milli-Q H_2O to 1 mL in a 2 mL tube. Prepare in duplicate.

Prepare working Micro BCA reagent at a ratio of 25 A: 24 B: 1 C. (i.e. for 30 mL of working reagent, add 15 mL A: 14.4 mL B: 0.6 mL C).

Add 1 mL of working Micro BCA reagent to each 1 mL albumin standard or sample. Mix and incubate at 60 ^{o}C for 1 h.

Allow to cool to room temperature. Transfer to disposable cuvettes (Materials).

Record the absorbance at 562 nm using a spectrophotometer (Materials). Use Milli-Q H_2O as reference.

Plot absorbance values (y) as a function of protein concentration (x) for the albumin standards. Plot the linear regression (y=mx+c) and rearrange the function (x=(y-c)/m) to determine the protein concentration (x) for each diluted sample measurement. Factor the diluted measurement to the undiluted sample concentration and take the average concentration of the duplicate measurements.

3. Materials

3.1 Chemical reagents

- Micro BCA Protein Assay kit (Pierce 23235)

3.2 Equipment

- Heat block
- Disposable cuvettes semi-micro PS (Fisherbrand FB55147)
- Ultrospec 3100 pro UV/Visible Spectrophotometer (Amersham Biosciences)
- Microsoft Excel

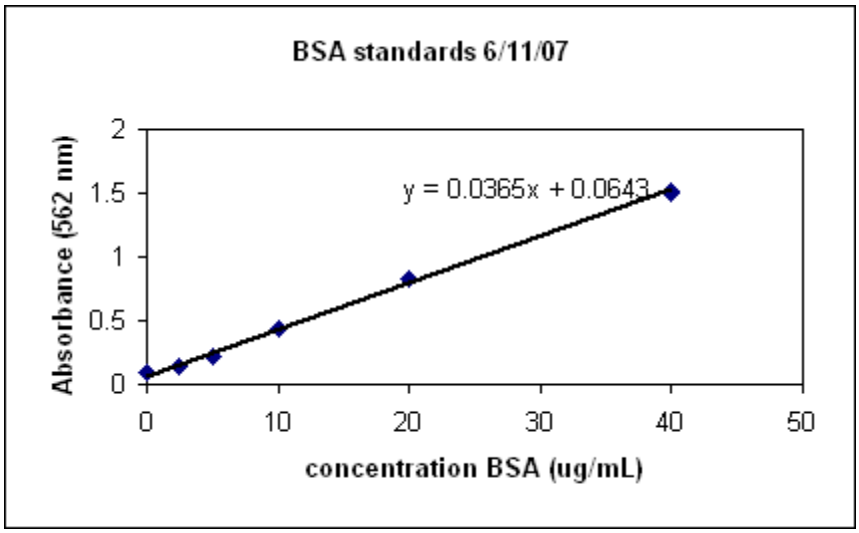
4. Quality Control

Absorbance measurements taken for the duplicate standard or sample dilutions that differ by greater than 0.1 units are discarded. Duplicate measurements are normally within a range of ~ 0.03 units. Samples to be compared on a western blot should be quantitated together to eliminate the effects of day to day variation in the Micro BCA assay. Typically, the protein concentration of a 1 mL hippocampal extract preparation is ~3 mg/mL.

5. Example Data

Hippocampal lysates DOC extracted on 13.7.07 & 5.10.07 HRAS (B6)

ug/mL	A(562)
0	0.083
0	0.087
2.5	0.144
2.5	0.141
5	0.221
5	0.222
10	0.436
10	0.442
20	0.826
20	0.826
40	1.518
40	1.503



diluted ug/mL	A(562)	Sample#	Sample name	uL	mg/mL	avg mg/mL	Dilute **150**uLaliquot to 1mg/mL with uL 2X SB
18.73	0.748	1	wt 69933	5	3.75		
19.14	0.763	1	wt 69933	5	3.83	3.79	418.11
18.18	0.728	2	wt 69935	5	3.64		
18.32	0.733	2	wt 69935	5	3.66	3.65	397.56
16.43	0.664	3	homo 69931	5	3.29		
17.47	0.702	3	homo 69931	5	3.49	3.39	358.52
17.17	0.691	4	homo 69932	5	3.43		
16.59	0.67	4	homo 69932	5	3.32	3.38	356.47
16.87	0.68	5	homo 91358	5	3.37		
17.28	0.695	5	homo 91358	5	3.46	3.41	362.22

17.22	0.693	6	homo 91356	5	3.44		
17.36	0.698	6	homo 91356	5	3.47	3.46	368.79
17.06	0.687	7	wt 91357	5	3.41		
17.75	0.712	7	wt 91357	5	3.55	3.48	372.08
18.65	0.745	9	wt 81999	5	3.73		
18.43	0.737	9	wt 81999	5	3.69	3.71	406.19

6. Supporting information

7. Document History

This document created by Rachel T. Uren on 22 February 2008.