

## Western Blot Analysis: Protein Quantification

Section of Cancer Genomics, Genetics Branch, NCI  
National Institutes of Health

### Reagents

#### BCA Protein Assay Reagent Kit

Pierce, Cat. 23225

### Preparation

#### Lysis Buffer + Protease Inhibitors (PIH)

See "Protein Extracts for Westerns"

#### BSA Standards

Make standards by diluting supplied BSA [2 mg/ml] into appropriate Lysis Buffer + PIH as in the following chart:

Tube Name	Volume of BSA	From Tube	Volume Lysis Buffer	Final [BSA]
	30.0 $\mu$ l of	STOCK	0 $\mu$ l	2.00 mg/ml
A	37.5 $\mu$ l of	STOCK	12.5 $\mu$ l	1.50 mg/ml
B	32.5 $\mu$ l of	STOCK	32.5 $\mu$ l	1.00 mg/ml
C	17.5 $\mu$ l of	A	17.5 $\mu$ l	0.75 mg/ml
D	32.5 $\mu$ l of	B	32.5 $\mu$ l	0.50 mg/ml
E	32.5 $\mu$ l of	D	32.5 $\mu$ l	0.25 mg/ml
F	32.5 $\mu$ l of	E	32.5 $\mu$ l	0.125 mg/ml
G	10.0 $\mu$ l of	F	40.0 $\mu$ l	0.05 mg/ml

### Procedure

1. Thaw extracts on ice.
2. Use Pierce BCA kit, following directions inside.
3. Make 1:10 dilutions of each cell lysate into Lysis Buffer + PIH and from these mix 1  $\mu$ l diluent into 4  $\mu$ l Lysis Buffer + PIH for a final dilution of 1:50.

4. Make working solution (WS) by combining 2500  $\mu$ l Soln. A + 50  $\mu$ l Soln. B from BCA Protein Assay Reagent Kit.
5. Mix 5  $\mu$ l each standard and 5  $\mu$ l each 1:50 dilution with 95  $\mu$ l working solution for a final dilution of 1:20.
6. Incubate at 37°C for 30 min.
7. Place tubes in ice/water slurry to inhibit reaction while reading absorption at 562nm.