Western Blotting

After determining cell lysate concentration, lysates (total of 50 ug protein) were mixed with sample buffer (two volumes) and heated on the heat block at 90 C for 10 min. Centrifuged, put on ice and loaded on gel.

SDS-PAGE Running Buffer (Towbin)- 2 L

25 mM Tris, 192 mM glycine, 0.1% SDS

1X Running Buffer		10X Running Buffer	
Reagents needed:		**Reagents needed:**	
28.8 g	glycine	288 g	glycine
6.04 g	Tris base	60.4 g	Tris base
2 g	SDS	20 g	SDS
1.8 L	ddH_2O	1.8 L	ddH_2O

**** CAUTION ** SDS powder is hazardous. Prepare solution in a ventilated fume hood.**

Directions:

1) Dissolve Tris base and glycine together in 1.8 L of ddH_2O.
2) Add SDS and mix.
3) Add ddH_2O to a final volume of 2 L.

Transfer buffer.

25 mM Tris, 192 mM glycine, 10% methanol

1X Transfer Buffer		10X Transfer Buffer	
Reagents needed:		**Reagents needed:**	
28.8 g	glycine	288 g	glycine
6.04 g	Tris base	60.4 g	Tris base
200 ml	methanol	-	methanol
1.6 L	ddH_2O	1.8 L	ddH_2O

** NOTE: for the proper transfer of large proteins, up to 0.5% SDS may need to be added to 1X Transfer Buffer. **

Directions for 1X Transfer Buffer:

1) Dissolve Tris base and glycine together in 1.6 L of ddH_2O.
2) Add methanol and mix.
3) Add ddH_2O to a final volume of 2 L.

Directions for 10X Transfer Buffer:

1) Dissolve Tris base and glycine together in 1.8 L of ddH_2O.
2) Add ddH_2O to a final volume of 2 L.

** To make 1X Transfer Buffer from 10X: Mix 100 ml of 10X Transfer Buffer, 100 ml of methanol and 800 ml of ddH_2O per liter **

Membrane blocking: blocker non-fat dry milk (1g) in 1X Tris buffered saline (10ml, 1X TBS) + 0.1% Tween 20. After blocking (1 h), membrane washed with 1X Tris for 10 min to prepare for antibody.