High Throughput Western Blotting Protocol for Antibody Quality Control

SDS-PAGE

Samples**:** Lysates from all 60 cell lines of the NCI-60 human cancer cell lines are pooled together in equal amounts

Sample preparation for electrophoresis:

- 5.5ul sample lysates (~10-20ug) + 159.5ul loading buffer for each prep-well gel
- broad range ladder
- 4-15% gradient gel

Loading: for each gel, load 8ul of ladder in small well and 165ul of sample in prep well

Run: 1hr at 150V

TRANSFER

Preparation:

- Transfer Buffer at 4C
- Nitrocellulose membrane = 0.45um
- Insert gel sandwich into transfer unit
- Place cooling device in transfer unit

Run 1hr at 100V

Check transfer with Ponceau S

- Pour enough Ponceau S on membrane to completely cover it
- Let stand 3-5 minutes, then wash off gently with tap water
- Proteins should be visible, appearing as red/pink stains

Mark membrane for cutting

- Measure out 4mm intervals across top edge of stained membrane
- Using a razor, cut off and discard unstained (no protein) membrane from all edges

Wash membrane in Wash Buffer 2 x 10min, 2 x 5min

Cut membrane into strips

- Cut membrane at each 4mm mark, parallel to the ladder

IMMUNOSTAINING

Block

- Place each strip in a separate mini-chamber with 500ul blocking buffer
- Incubate 1hr at RT or O/N at 4C

Primary antibodies

- Add each antibody directly to each mini-chamber with blocking buffer and strips
- Dilutions vary per antibody
- Incubate 1hr at RT or O/N at 4C

Wash strips

- Keep strips in mini-chambers; discard the solution (blocking buffer + primary antibody)
- Wash in wash buffer 2 x 10min, 2 x 5min

Secondary antibody

- Prepare working solution of 1:50, 000, using blocking buffer and HRP-conjugated goat anti-mouse
- Add 500ul of working solution to each mini-chamber
- Incubate 1hr, RT

Wash in wash buffer 2 x 10min, 2 x 5min

Do not allow strips to dry out

DETECTION

Reassemble membrane

Line up all strips in same order as on original intact membrane

Detection system

Pierce SuperSignal West Pico Chemiluminescent Substrate, *or*
Pierce SuperSignal West Femto Maximum Sensitivity Substrate
Incubate, as per included instructions
Remove excess liquid

Develop

Place development folders in cassette
In darkroom, expose strips with imaging film as needed (begin with 60sec) and develop
Do not attempt to expose film less than 5 seconds

SOLUTIONS AND REAGENTS

Sample Loading Buffer
62.5mM Tris-HCl, pH 6.8
2% SDS
10% glycerol
2.5% BME, added just before loading samples into gel

Wash Buffer (500ml)
500mL PBS
0.5mL Tween-20

Transfer Buffer (1000ml, chilled)
100mL 10x Tris/glycine transfer buffer
200mL methanol
700mL dH2O

Blocking Buffer (500mL)
500mL PBS
1.0g I-Block^{tm} (Tropix, Bedford, MA, USA), dissolved by heating/stirring
0.5mL Tween-20

Running Buffer
10x Tris/glycine/SDS buffer (BioRad)

Other Supplies
Nitrocellulose membrane, 0.45um (BioRad)
Development folders (Tropix, Bedford, MA, USA)
Film (Kodak ??? please check specs)

Ponceau S
Please see back page(s) of my lab notebook for recipe!