WESTERN BLOTTING USING ANTIBODIES AGAINST HISTONE PROTEINS

The following protocol refers to the Western blot detection of histone proteins derived from purified calf thymus performed at Abcam.

1. For each lane prepare 0.5µg calf thymus or acid extracted histones diluted in 1X NuPAGE LDS sample buffer (Invitrogen) supplemented with 100mM DTT. Heat the sample to 95°C for 5 minutes. Centrifuge the sample briefly to restore sample volume from condensation formed in the tube during heating.

2. Prepare a 10% NuPAGE Bis Tris gel 1.0mm. A higher percentage gel (15%) is recommended for more effective resolution of histone proteins.

3. Load the histone samples, remembering to include a pre-stained protein standard (Precision Plus Protein Standard (Kaleidoscope), Bio-Rad). Run the gel in NuPAGE MES SDS running buffer at 200V for 35mins. NB It is advisable not to run the dye front completely off the gel.

4. Transfer the protein samples onto a nitrocellulose membrane with reduced pore sizes (Invitrogen; LC2000) at 30V for 70 minutes using NuPAGE transfer buffer (1X)/ 20% methanol.

5. Verify the successful transfer and equal loading of the histones using Ponceau staining. Dilute the Ponceau out of the membrane by adding dH_2O.

6. Block the membrane for 1 hour at room temperature (RT) using 5% BSA / 0.1% TBST (50mM Tris-HCl, pH 7.5, 150mM NaCl, 0.1% Tween 20).

7. Cut the membrane into strips if necessary and prepare the primary antibody by diluting in blocking buffer (5% BSA / 0.1% TBST) at a dilution recommended by the Abcam datasheet. Add blocking peptides (see Blocking peptide protocol) as required and incubate on a rotating platform for 20 minutes at RT. Incubate the membrane with the primary antibody for 1.5 hours at RT or overnight at 4°C.

8. Rinse the blots briefly in 0.1% TBST and then perform two 5 minute washes followed by two 10 minute washes using the same buffer.

9. Incubate the membrane with the secondary antibody for 1 hour at RT, diluted in 5% BSA / 0.1% TBST. (For example ab6721: Goat polyclonal to rabbit IgG H&L (HRP)).

10. Wash the membrane in 0.1% TBST twice for 5 minutes, and twice for 10 minutes.

11. Add ECL reagents for 3 minutes at RT. Capture WB image using Syngene GeneGnome using various durations of exposure: 10sec, 30sec, 1min, 2mins, 3mins, 4mins and 5mins.

Top Tips for Successful Western blotting with our range of Histone antibodies.

- *Use a high percentage gel for clear resolution of histone proteins.*
- *Use a nitrocellulose membrane with a pore size of 0.2µm to ensure optimal capture of histone proteins.*
- *Use high quality BSA in your blocking solutions rather than conventional dried milk such as Marvel.*