Histone Immunoblotting Protocol

Histones are basic nuclear proteins that are responsible for the nucleosome structure of the chromosomal fiber in eukaryotes. Nucleosomes consist of approximately 146 bp of DNA wrapped around a histone octamer composed of pairs of each of the four core histones (H2A, H2B, H3, and H4). The chromatin fiber is further compacted through the interaction of a linker histone, H1, with the DNA between the nucleosomes to form higher-order chromatin structures.

Successful immunoblotting of histones must overcome an important consequence of histone composition, namely, their relatively low molecular weight. The molecular weight of histones affects both resolution of histone proteins in SDS-PAGE and their efficient transfer to nitrocellulose membrane. Good results are achieved by using high percent agarose gels and nitrocellulose with a pore size of 0.2 µm to ensure optimal capture of histone proteins. We also suggest using high-quality BSA in the blocking solutions rather than conventional dried milk.

I. Reagents Required

Reagent	Preparation
Membrane Blocking Buffer	% (w/v) BSA (BSA-10) with 0.2% (v/v) Tween-20 dissolved in TBS or PBS as preferred. Often 10X TTBS (MB-013) 10X TTBS pH 7.5 (1.0 M Tris HCl 1.5 M Sodium Chloride 0.1% (w/v) Tween-20) or 10X PBS (MB-008) 10X PBS pH 7.2 (0.2 M Potassium Phosphate 1.5 M Sodium Chloride) is used
Membrane Wash Buffer	0.2% (v/v) Tween-20 in TBS or PBS as preferred

II. Procedure

1. Run sample on high percentage (%T/%C) SDS-PAGE gel to resolve histone proteins.
2. Transfer and immobilize antigen on nitrocellulose membrane with a pore size of 0.2 µm to ensure optimal capture of histone proteins.
3. Block the membrane with membrane blocking buffer for 1 hour at room temperature.
4. Dilute the primary antibody in blocking buffer and incubate the blots overnight at 4°C or 2–3 hour at room temperature with gentle rotation. You may have to determine serial dilutions of primary antibody to establish the optimal dilution in your system. For blocking peptide studies, a final peptide concentration of 0.1 to 1.0 µg/mL peptide should be pre-incubated with the antibody for around 20 minutes at room temperature before the antibody is added to the blots. Incubate peptide-blocked antibody with blot as above.
5. Wash the blots with three 5-min washes in membrane wash buffer.
6. Prepare the appropriate secondary antibody conjugate in blocking buffer and incubate the blots for 1 hour at room temperature with gentle rotation.
7. Wash the blots with three 5-min washes in blocking buffer and then perform a final rinse in PBS or TBS.
8. Perform *Max*Tag™, ECL, ECF, IRDYE800™ (for LI-COR ODYSSEYÓ) detection or equivalent method to visualize the blot.

III. Notes

For best results, let the gel polymerize for 1 hour then pre-run the gel for 1 hour using a constant current of ~20 milliamps. Typically, 2 L of 1X TGE is used: 1.5 L in the bottom reservoir and 0.5 L in the top reservoir when using a commercially available apparatus. Do not exceed a final concentration of 100 mM sodium chloride in the reaction mixture. Concentrations above 100 mM inhibit the reaction. Do not exceed 2.5 mL of nuclear extract per reaction mixture. Specific antibodies/probes may require altered conditions, for instance, NF-Y antibodies must be incubated for 1–2 hours on ice before adding the probe.Prepare the reaction mixture in duplicate using unlabeled (cold) probe as a negative control or add cold probe and incubate 10minutes at room temperature before adding labeled probe for competition experiments. Certain gel super shift antibodies are supplied with control peptides. Prepare these reaction mixtures in duplicate adding the control peptide to the reaction mixture prior to adding the antibody.

References

Gel Electrophoresis of Proteins: A Practical Approach. Third Edition. Ed. B.D. Hames. Oxford University Press. Oxford, UK. 1998