www.rockland.com
tech@rockland.com
+1 484.791.3823

Protocol

Histone Immunoblotting

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 the 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.

Reagents Required

Product	Preparation	Item No.
Membrane Blocking Buffer	% (w/v) BSA with 0.2% (v/v) Tween-20 dissolved in TBS or PBS as preferred. Often 10X TTBS pH 7.5 (1.0 M Tris HCl 1.5 M Sodium Chloride 0.1% (w/v) Tween-20) or 10X PBS pH 7.2 (0.2 M Potassium Phosphate 1.5 M Sodium Chloride) is used.	BSA-10 / MB-013 / MB-008
Membrane Wash Buffer	0.2% (v/v) Tween-20 in TBS or PBS as preferred.	

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 hours 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-minute 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 MaxTag™, ECL, ECF, IRDYE800™ (for LI-COR ODYSSEY®) detection or equivalent method to visualize the blot.

References

- Hames, B.D. (1998). Gel Electrophoresis of Proteins: A Practical Approach. Third Edition. Oxford University Press