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NB100-123 Protocol

Western Blot protocol for HIF-1 alpha Antibody (NB100-123)

[[URL:https://www.novusbio.com/products/hif-1-alpha-antibody-h1alpha67_nb100-123]][[Caption:HIF-1 alpha Antibody]]

Western Blot Protocol

1. Resolve aliquots (25-30 ug) of induced nuclear protein extracts on a 4-20% Tris-HCl gel.
2. Transfer proteins to nitrocellulose membrane in 20 mM Tris-HCL (pH 8.0)/150 mM glycine/20% (vol/vol) methanol.
3. Block membrane for 1 hour with 1X western wash buffer containing 5% non-fat dry milk (NFDM).
4. Incubate membrane overnight at 4C in NB 100-123 diluted in 1X western wash/5% NFDM.
5. Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
6. Incubate membrane with HRP conjugated anti-mouse IgG for 1 hour (RT) in 1X western wash/5% NFDM.
7. Wash with 1X western wash for 35 minutes at RT (1 X 15 minutes, 2 X 10 minutes).
8. Drain membrane and place on saran wrap.
9. Using Amersham ECL Kit, mix equal volumes of two reagents. Pour over membrane (protein side facing up). Let solution sit on membrane for 15-20 seconds.
10. Drain membrane and place on new saran wrap.
11. Wrap up membrane and expose to film.
12. Develop accordingly.

10X Western wash: 24.2 g Tris, 80g NaCl, Tween-20 to 1%, pH 7.6 and QS to 4L.
Stripping buffer: 100 mM BME 2% SDS 62.5 mM Tris (pH 6.7)
To strip membrane: Incubate membrane in stripping buffer for 30 minutes at 56C. Wash membrane for 15 minutes with several change of 1X western wash.

Notes: If hypoxia treatment is not hypoxic enough (less than 2% oxygen to get an induction), signal will be absent. Also, if the harvest time is too slow or there are not enough protease inhibitors, etc., the induced protein will be rapidly lost as HIF-1alpha has a very short half-life.
Nuclear Extract Preparation Reference: Wang and Semenza. Purification and Characterization of Hypoxia-Inducible Factor. Journal of Biological Chemistry. 270(3): 1230-1237, 1995.

**This antibody has demonstrated varying results in Western blot applications. Product NB100-105 is recommended for most Western blot experiments.