

NB100-124 Protocol

Protocol specific for HIF-1 beta Antibody (NB100-124)

ARNT/HIF-1 beta Antibody (H1beta234): https://www.novusbio.com/products/arnt-hif-1-beta-antibody-h1beta234_nb100-124
Western Blot Procedure

1. Resolve aliquots (15 mg) of induced nuclear protein extracts on a SDS/6% polyacrylamide gel.
2. Transfer to nitrocellulose membranes in 20 mM Tris-HCl (pH 8.0)/150 mM glycine/20% (vol/vol) methanol.
3. Block membranes for 1.5 hours with 1X western wash buffer containing 5% non-fat dry milk (NFDM).
4. Incubate membranes for 1.5 hours at room temperature (RT) in NB 100-124 diluted 1:1,500 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 membranes 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

24.2g 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)

Incubate membrane for 30 minutes at 56 degrees C. Wash membrane 15 minutes with several changes 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-1beta has a very short half-life. Whole cell extracts or nuclear extracts of hypoxia induced cell lines (293, Hep3B, COS7, Hepa) are useful as a positive control. Nuclear Extract

Preparation Reference: Wang and Semenza. "Purification and Characterization of Hypoxia-Inducible Factor 1". Journal of Biological Chemistry. 270(3): 1230-1237, 1995.