

NB200-174 Protocol

Western Blot protocol specific for p19ARF Antibody (NB200-174)

[[URL:https://www.novusbio.com/products/p19arf-cdkn2a-antibody-5-c3-1_nb200-174]][[Caption:p19ARF Antibody]]
Western Blot

1. Cells are lysed by addition of RIPA buffer and incubated on ice for 20 minutes, with occasional agitation.
2. Insoluble material is pelleted by centrifugation at 20,000 RPM for 15 min at 4C.
3. Protein in the supernatant is quantified by BCA assay.
4. Proteins are separated on a 12% SDS-denaturing gel.
5. Proteins are then transferred to PVDF membranes.
6. Membranes are probed for 1 hour with primary anti-p19ARF [cat# NB 200-174], diluted in TBS-T containing 10% nonfat milk powder.
7. The membrane is then washed several times with TBS-T.
8. The membrane is incubated for 30 minutes in a secondary antibody to rat IgG conjugated to horseradish peroxidase (HRP), diluted in TBS-T containing 5% milk.
9. After several washes with TBS-T, antibodies are visualized by incubation with Western Lightning Chemiluminescent Reagent (Perkin Elmer Life Sciences, Boston, MA), followed by autofluorography.

Buffers

- RIPA:50 mM Tris,pH 8.0
- 150 mM NaCl
- 1% Triton X-100
- 0.5% sodium deoxycholate
- 0.1% SDS
- 1 mM phenylmethylsulfonyl fluoride [PMSF]
- 0.3 units/mL Aprotinin
- 10 mM beta-glycerophosphate
- 1 mM NaF
- 0.1 mM NaVO₄

TBST:

- 10 mM Tris, pH 7.4
- 150 mM NaCl
- 0.1%Tween-20

Western blot

1. Total cell lysates (25 ug) from NIH3T3 cells, which have deleted the Arf gene and from wild type mouse embryo fibroblasts (MEFs) at passage 6, which express p19Arf, were resolved by SDS-PAGE.
2. Electroblotted onto a PVDF membrane.
3. Blocked with 10% non-fat milk powder in TBS-Tween.
4. The membrane was then probed with 1ug/ml of purified anti-p19Arf (NB 200-174).
5. The membrane was then probed with an anti-rat IgG-HRP secondary.
6. p19Arf was visualized using a chemiluminescent reagent.