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NB200-169 Protocol

Protocol specific for p19ARF Antibody (NB200-169)

p19ARF/CDKN2A Antibody (12-A1-1): https://www.novusbio.com/products/p19arf-cdkn2a-antibody-12-a1-1_nb200-169
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 4 degrees Celcius.
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 200-169], 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 glycerophosphate 1 mM NaF 0.1 mM NaVO4 TBS-T: 10 mM Tris, pH 7.4 150 mM NaCl 0.1%Tween-20