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## NB200-111 Protocol

# Protocol specific for p14ARF Antibody (NB200-111)

[[URL:https://www.novusbio.com/products/p14arf-cdkn2a-antibody_nb200-111]][[Caption: p14ARF Antibody]]
Western Blot Procedure

1) Lyse HeLa or BT549 cells in Laemli buffer (2% SDS, 62.5 mM Tris pH 6.8, 10% glycerol, 5% 2-mercaptoethanol).

2) Boil the lysate for 5 minutes.
3) Load 25 ug of total cell extract, per lane, in a 15% SDS-PAGE minigel.
4) Transfer protein to PVDF membrane in 40 mM Tris base, 20 mM NaAcetate, 2 mM EDTA pH 7.4, 0.05% SDS, 20% methanol for 1 hour at 4 degrees C.
5) Rinse membrane in PBS-T and then block membrane in 5% nonfat dry milk/PBS-T (0.01M phosphate, 0.0027M KCl, 0.137M NaCl pH 7.4, 0.1% Tween-20) for 1 hour at room temperature or overnight at 4 degrees C.
6) Incubate membrane overnight at 4 degrees C with properly diluted NB200-111 (see data sheet) in PBS, 0.2% Tween-20, 5% nonfat dry milk.
7) Rinse the membrane 2X with 40 ml PBS-T. Wash membrane at room temperature, with 40 ml of PBS-T, 1X for 15 minutes and 2X for 5 minutes, each.
8) Incubate membrane with HRP conjugated anti-rabbit, diluted in PBS-T with 5% nonfat dry milk, for 1 hour at room temperature.
9) Wash membrane at room temperature, with 40 ml of PBS-T, 1X for 15 minutes and 4X for 5 minutes, each.
10) Develop with ECL reagents (Amersham) and autoradiography. Expose for 1+ minutes. For BT549 cells, 1 minute exposure is needed. For HeLa cells, additional exposure time may be required. In addition, a stronger ECL (Pierce) may be necessary.

NOTE: HeLa whole cell extracts (NB800-PC1) were used as a positive control for this antibody.