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NB200-179 Protocol

Western Blot Protocols specific for 15-PGDH Antibody (NB200-179)

[[URL:https://www.novusbio.com/products/15-pgdh-hpgd-antibody_nb200-179]][[Caption:15-PGDH Antibody]]
Western Blot I (LoVo lysates)

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 20ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transferapparatus.
3. Stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk in TBS for 1 hour.
6. Dilute the rabbit anti-15-PGDH primary antibody (NB 200-179) in blocking buffer and incubate overnight at 4C.
7. Wash the membrane in water for 5 minutes and apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
8. Wash the blot in TBS containing 0.05-0.1% Tween-20 for 10-20 minutes.
9. Wash the blot in type I water for an additional 10-20 minutes (this step can be repeated as required to reduce background).
10. Apply the detection reagent of choice in accordance with the manufacturers instructions (Amersham's ECL is the standard reagent used at Novus Biologicals).

**Note: Tween-20 can be added to the blocking buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

Western Blot II (A549 lysates)

1. Cell lysates are prepared in RIPA buffer (50 mM 50 mM Tris-HCL/1% Nonidet P-40/0.25% Na-deoxycholate/150 mM NaCl/1 mM EDTA/1 mM PMSF) supplemented with a protease inhibitor mixture (Roche Applied Sciences).
2. They are then separated on 10% or 12% SDS/PAGE (30-150 ug per lane) and transferred to a Immobilon PVDF membrane (Millipore).
3. The membrane is blocked with 5% NFDM in TBS-T (TBS + 0.1% Tween-20).
4. The membrane is then probed with the diluted anti-PGDH antibody (NB 200-179), diluted in blocking buffer at RT for 1 hour.
5. The membrane is washed 3 times with TBS-T.
6. The membrane is then incubated with a biotinylated goat anti-rabbit IgG (diluted as per manufacturer's guidelines in blocking buffer) at RT for 1 hour.
7. The membrane was washed extensively.
8. The membrane is then incubated with an HRP-conjugated streptavidin (1:2,000) complex at RT for 1 hour.
9. The membrane is washed extensively.
10. The membrane is developed using and ECL detection system.