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Certificate of Analysis

Anti-p75NTR (Neurotrophin Receptor), clone ME20.4

(mouse monoclonal IgG_1)
Catalog # 05-446
Lot # 2484171

Immunogen: Human melanoma cell line WM245. Hybridoma Clone ME20.4

Specificity: Recognizes the low affinity p75NTR, Mr 69kDa.

Species Cross-reactivity: Human and monkey.

Formulation: **100µg** of protein G purified mouse IgG_1 in **132µl** of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at –20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap.

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

Quality Control Testing

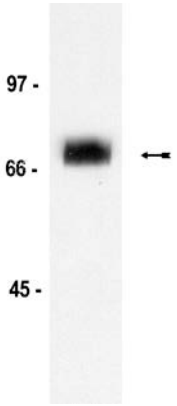
Immunoblot Analysis: 0.1-1µg/ml of this lot detected p75NTR in RIPA lysates from HS294T cells.

Immunoprecipitation: 4µg of a previous lot immunoprecipitated p75NTR from 500µg of HS294T RIPA lysate.

Additional Research Applications

Immunohistochemistry: Reported to detect p75NTR in tissue section. Also used in immuno-electron microscopy.

Biological Blockade: Reported to block ^{125}I-labeled NGF binding to its receptor.



Immunoblot Analysis
Representative blot from a previous lot. HS294T cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-p75NTR (1µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates p75 NTR (69kDa).

Application References:

1. Ross, A.H., *et al*., Proc. Natl. Acad. Sci. USA **81:** 6681-6685, 1984.
2. Dominici,C., J. Neurooncol. **31:** 57-64, 1997.
3. Wakabayashi,Y., Neurosci. Lett. **186:** 9-12, 1995.
4. Caneva,L., Blood Cells Mol. Dis. **21:** 73-85, 1995.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1μg/ml each aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.1-1μg/ml of anti-p75NTR (Neurotrophin Receptor),** diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-mouse** HRP conjugated IgG, Catalog # 12-349, 1:2000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1μg/μl total cell protein in a microcentrifuge tube with PBS.
2. Add **4μg of anti-p75NTR (Neurotrophin Receptor)** to 500μg-1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100μl (50μl packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 60μl 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or boiled for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.