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Certificate of Analysis

Anti-TGFβR Type II

(rabbit polyclonal IgG)
Catalog # 06-227
Lot # 1993905

Immunogen: Rabbit polyclonal serum generated against a synthetic 28 residue peptide conjugated to keyhole limpet hemocyanin. The peptide corresponds to the first 28 N-Terminal residues of the mature human TGFβ receptor.[2]

Specificity: Specific for the p75 TGFβ Type II receptors. Does not cross-react with other identified receptors. Cross-reactivity with rodent isoforms has been observed.

Formulation: **400µg** packaged as two vials, each vial containing **200µg** of protein A-purified rabbit IgG in **200µl** 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide.

Storage and Stability: Stable for 2 years at 2°-8°C from date of shipment.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

Note: The TGFβR Type II is a low abundant protein, approximately 200 receptors/cell.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing and Research Applications

Immunoblot Analysis: 2-4µg/ml detected the p75 TGFβR Type II in a TF-1 cell lysate. Previous lots detected the p75 TGFβR Type II in WI-38 human diploid fibroblasts and a rat L6 cell lysate using chemiluminescence.

Immunoprecipitation: Use 1-5µg/sample to immunoprecipitate p75 TGFβR Type II from lysates of [^{35}S] labeled cells.

Immunocytochemistry: Use 5-10µg/ml in formalin-fixed paraffin embedded tissue.

Application References:

1. Chen, D., *et al.,* Mol. Cell. Biol. **19**: 4684-4694, 1999.
2. Lin, *et al.,* Cell 68: 775-785, 1992.
3. Hall, F.L., *et al.*, Biochem. J. **316:** 303-310, 1996.
4. Yamada, K., *et al.*, Blood **90**: 4832-41, 1997.

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Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml 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) and 0.05% Tween® 20 (PBST-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **2-4µg/ml of anti-TGFβR Type II,** diluted in freshly prepared PBST-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-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution, was used) in PBST-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).

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