

Certificate of Analysis

Anti-Met

(rabbit polyclonal IgG)

Catalog # 07-283

Lot # 33422

Immunogen: Ovalbumin-conjugated, synthetic peptide corresponding to amino acids 1361-1379 (APYPSLLPSQDNIDGEGNT) of mouse c-Met. The immunizing sequence has 18 of 19 identical amino acids in rat.

Specificity: Recognizes the Met receptor precursor, Mr 170kDa and the c-Met β chain, Mr 140kDa. A non-specific protein was also detected, Mr 45kDa.

Species Cross-reactivity: Mouse. Predicted to cross-react with rat based on sequence homology.

Formulation: 200 μ g of protein A purified rabbit IgG in 200 μ l of 70% storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide) and 30% glycerol. Store at -20°C.

Storage and Stability: Stable for 2 years at -20°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.

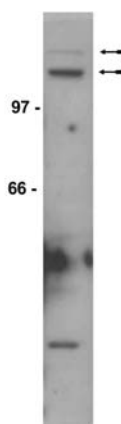
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 2-4 μ g/ml of this lot detected Met in mouse liver membrane fractions.

Additional Research Applications

Immunoprecipitation: This antibody has been reported by an independent laboratory to immunoprecipitate c-Met.



Immunoblot Analysis

Representative blot from a previous lot. Mouse liver membrane fraction was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Met (2 μ g/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicate the Met receptor precursor (~170kDa) and the c-Met β chain (~140kDa).

General References:

1. Borset, M., *et al.*, Leuk. Lymphoma **32**: 249-256, 1999.
2. Moriyama, T., *et al.*, Int. J. Mol. Med. **3**: 531-536, 1999.
3. Birchmeier, C., and Gherardi, E., Trends Cell Biol. **8**: 404-410, 1998.

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; 1 μ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200) and 0.05% Tween[®]-20 (TBST-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **2-4 μ g/ml of anti-Met**, diluted in freshly prepared TBST-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 TBST-MLK for 1 hour at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-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).