

Certificate of Analysis

Anti-Met

(rabbit polyclonal IgG)

Catalog # 07-283

Lot # 22164

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 Met, doublet at Mr 170 and 140kDa. A non-specific protein was also detected at ~45kDa.

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

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

Formulation: 200mg of protein A purified rabbit IgG in 200ml 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.

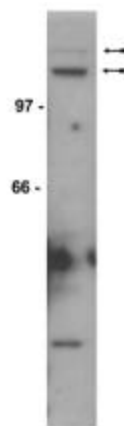
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 2µg/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

Mouse liver membrane fraction was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Met (2µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrows indicates 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 EGTA; 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 5% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween 20 (TBST-MLK) for 60 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **2mg/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).