

Certificate of Analysis

Anti-phospho-Met (Y1234, Y1235)

(rabbit immunoaffinity purified IgG)

Catalog # 07-211

Lot # 20438

Immunogen: KLH-conjugated, synthetic peptide (DKE[pY][pY]SVHMK-C) corresponding to amino acids 1231-1240 of human phospho-Met/HGFR. The sequence is identical in mouse, rat, sheep, *Xenopus*, chicken, and puffer fish.

Specificity: Recognizes phosphorylated Met, Mr 140kDa.

Species Cross-reactivity: Human and mouse. Other species have not been tested.

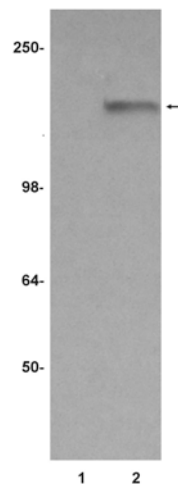
Storage and Stability: Stable for 1 year at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

Formulation: 200µl of immunoaffinity purified rabbit polyclonal IgG in 0.05% sodium azide and 5mg/ml BSA 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: 1:250-1:1000 of this lot detected pMet in RIPA lysates from NIH-3T3 cells co-expressing MET/HGFR and HGF.



Immunoblot Analysis

RIPA lysates of NIH-3T3 cells co-expressing MET/HGFR and HGF were resolved by electrophoresis and transferred to nitrocellulose. The nitrocellulose was treated with Yersinia Protein Tyrosine Phosphatase (lane 1) or untreated (lane 2) and then probed with anti-phospho-Met (1:250). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Met.

General References:

- Rodrigues, G.A., *et al.*, *Oncogene* **9**: 2019-2027, 1999.
Rerracini, R., *et al.*, *J. Biol. Chem.* **29**: 19558-19564, 1991.
Naldini, L., *et al.*, *Mol. Cell. Biol.* **4**: 1793-1803, 1991.

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; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. If desired, perform YOP treatment of immunoblot (see below) to verify primary antibody specificity.
3. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (TBS-MLK) for 20 minutes at 20-25°C with constant agitation.
4. Incubate the nitrocellulose with **1:250–1:1000 of anti-phospho-Met (Y1234, Y1235)**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
5. Wash the nitrocellulose twice with water.
6. 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 TBS-MLK for 1.5 hours at room temperature with agitation.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice (Biowest Extended Duration peroxidase substrate [UVP™ Inc.]).

Yersinia Protein Tyrosine Phosphatase (YOP) Treatment of Immunoblots

1. Block nitrocellulose membrane with 3%BSA in TBS for 1 hour at room temperature.
2. Place membrane strip in a 5 or 15ml screw-cap tube containing 1 ml YOP buffer (25mM HEPES, pH 7.3, 5mM EDTA, 10 mM 2-mercaptoethanol)
3. Add 180 Units Yersinia Protein Tyrosine Phosphatase (YOP), Catalog # 14-229, to 1ml YOP buffer containing 100µg/ml BSA.
4. Add the diluted phosphatase to the membrane strip and incubate at 30°C for 60 min.
5. Rinse nitrocellulose strip with water 3 times.
6. Continue at step 3 of the with immunoblot protocol above.