



cell signaling solutions

## Certificate of Analysis

10 Old Barn Road • Lake Placid, NY 12946  
Technical Support: T: 800 548-7853 • F: 518 523-4513  
email: techserv@upstate.com  
Sales Department: T: 800 233-3991 • F: 781 890-7738  
Licensing Dept.: 800 310-4659  
www.upstate.com

### Anti-phospho-Met (Y1234, Y1235)

(rabbit immunoaffinity purified IgG)

Catalog # 07-211

Lot # 25335

**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.

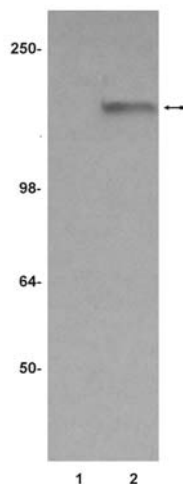
**Formulation:** 200 $\mu$ l of immunoaffinity purified rabbit polyclonal IgG in 0.2M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide and 5mg/ml BSA before the addition of glycerol to 30%. Liquid at -20°C.

**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.

**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

Representative blot from a previous lot. 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 (~140kDa).

### General References:

1. Rodrigues, G.A., *et al.*, Oncogene **9**: 2019-2027, 1999.
2. Rerracini, R., *et al.*, J. Biol. Chem. **29**: 19558-19564, 1991.
3. 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 EDTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 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 5% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 30-60 minutes at room temperature 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 (enhanced chemiluminescence was used).

### Yersinia Protein Tyrosine Phosphatase (YOP) Treatment of Immunoblots

1. Block nitrocellulose membrane in freshly prepared TBS containing 5% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 1 hour at room temperature.
2. Rinse the membrane three times with water.
3. Place membrane strip in a 5 or 15ml screw-cap tube containing 1ml YOP buffer (25mM HEPES, pH 7.3, 5mM EDTA, 10 mM 2-mercaptoethanol)
4. Add 180 Units Yersinia Protein Tyrosine Phosphatase (YOP), to 1ml YOP buffer containing 100 $\mu$ g/ml BSA.
5. Add the diluted phosphatase to the membrane strip and incubate at 30°C for 60 minutes with mild agitation. Be sure to set up an untreated control membrane in the same buffer without YOP.
6. Rinse nitrocellulose strip with water 3 times.
7. Block nitrocellulose for 30 minutes with TBS-MLK.
8. Continue at step 3 of the with immunoblot protocol above.