

Certificate of Analysis

Anti-phospho-Met (Tyr1234/1235)

(rabbit immunoaffinity purified IgG)

Catalog # 07-211

Lot # 30022

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: 200µl of immunoaffinity purified rabbit polyclonal IgG in 70% storage buffer (0.2M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide, 5mg/ml BSA) and 30% glycerol. Store at -20°C.

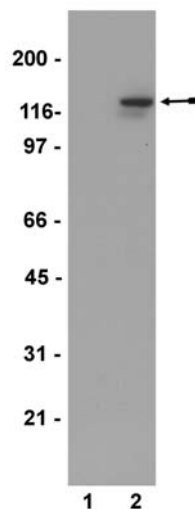
Storage and Stability: Stable for 1 year at -20°C from date of shipment.

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: 1:250-1:2000 of this lot detected phosphorylated Met in RIPA lysates from NIH-3T3 cells co-expressing MET/HGFR and HGF. Pretreating blots with 400U/ml of λ phosphatase prior to incubation with this lot of antibody abolished the detection of phospho-Met.



Immunoblot Analysis

RIPA lysates of NIH-3T3 cells co-expressing MET/HGFR and HGF were resolved by electrophoresis and transferred to nitrocellulose. Lane 1 was incubated with λ phosphatase while lane 2 was incubated without λ phosphatase before being probed with anti-phospho-Met (1:1000). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated Met (~140kDa).

Application References:

1. Chan, P. C., *et al.*, J. Biol. Chem. **277**: 50373-79. 2002.

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 with λ Phosphatase Treatment

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 for 1 hour in TBS containing 1% BSA and 0.1% Triton[®] X-100.
3. Add λ -phosphatase (Catalog # 14-405) to a final concentration of 400U/ml and DTT (Catalog # 20-265) to a final concentration of 5mM, to nitrocellulose and incubate overnight at room temperature with constant agitation.
4. Next day, rinse nitrocellulose with water and proceed with immunoblot protocol.
5. 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.
6. Incubate the nitrocellulose with **1:250-1:2000 of anti-phospho-Met (Tyr1234/1235)**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
7. Wash the nitrocellulose twice with water.
8. 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.
9. Wash the nitrocellulose with water twice.
10. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
11. Rinse the nitrocellulose in 4-5 changes of water.
12. Use detection method of choice (enhanced chemiluminescence was used).