Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF2480

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse HGF R/c-MET when phosphorylated at Y1234/Y1235 in Western blots.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human HGF R/c-MET Y1234/1235 sites
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS.

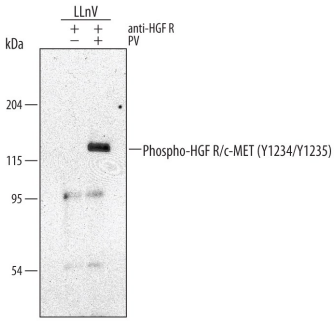
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
Simple Western	5 µg/mL	See Below
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

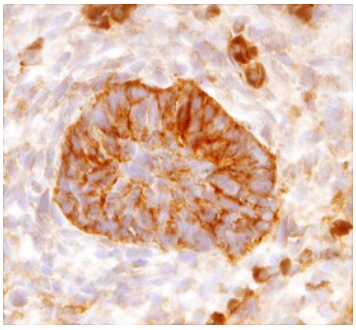
DATA

Western Blot



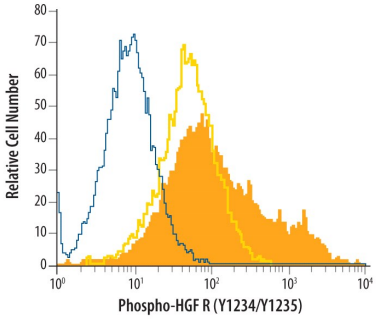
Detection of Human Phospho-HGF R/c-MET (Y1234/Y1235) by Western Blot. Western blot shows Goat Anti-Human HGF R/c-MET Antigen Affinity-purified Polyclonal Antibody (Catalog # AF276) immunoprecipitate of MDA-MB-468 human breast cancer cell line untreated (-) or treated (+) with 100 µM pervanadate (PV) for 10 minutes. PVDF membrane was probed with 0.5 µg/mL of Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-HGF R/c-MET (Y1234/Y1235) at approximately 145 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



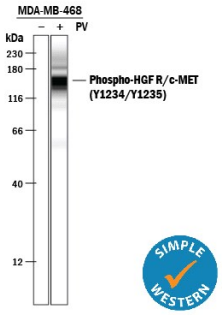
HGF R/c-MET in Mouse Embryo. HGF R/c-MET was detected in immersion fixed frozen sections of mouse embryo (13 d.p.c.) using Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480) at 15 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Rabbit HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS005) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Intracellular Staining by Flow Cytometry



Detection of HGF R/c-MET in pervanadate-treated MCF-7 Human Cell Line by Flow Cytometry. MCF-7 human breast cancer cell line was unstimulated (light orange open histogram) or treated with 100 µM pervanadate for 10 minutes (dark orange filled histogram), then stained with Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480), or control antibody (Catalog # AB-105-C, blue open histogram), followed by Phycoerythrin-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # F0110). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with methanol.

Simple Western



Detection of Human Phospho-HGF R/c-MET (Y1234/Y1235) by Simple Western™. Simple Western lane view shows lysates of MDA-MB-468 human breast cancer cell line untreated (-) or treated (+) with 100 µM Pervanadate (PV) for 10 minutes, loaded at 0.2 mg/mL. A specific band was detected for HGF R/c-MET at approximately 156 kDa (as indicated) using 5 µg/mL of Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF2480

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

HGF R, also known as Met (from *N*-methyl-*N*-nitro-*N*-nitrosoguanidine induced), is a glycosylated receptor tyrosine kinase that plays a central role in epithelial morphogenesis and cancer development. HGF R is synthesized as a single chain precursor which undergoes cotranslational proteolytic cleavage. This generates a mature HGF R that is a disulfide-linked dimer composed of a 50 kDa extracellular α chain and a 145 kDa transmembrane β chain (1, 2). The extracellular domain (ECD) contains a seven bladed β-propeller sema domain, a cysteine-rich PSI/MRS, and four Ig-like E-set domains, while the cytoplasmic region includes the tyrosine kinase domain (3, 4). Proteolysis and alternate splicing generate additional forms of human HGF R which either lack of the kinase domain, consist of secreted extracellular domains, or are deficient in proteolytic separation of the α and β chains (5-7). The sema domain, which is formed by both the α and β chains of HGF R, mediates both ligand binding and receptor dimerization (3, 8). Ligand-induced tyrosine phosphorylation in the cytoplasmic region activates the kinase domain and provides docking sites for multiple SH2-containing molecules (9, 10). HGF stimulation induces HGF R downregulation *via* internalization and proteasome-dependent degradation (11). In the absence of ligand, HGF R forms noncovalent complexes with a variety of membrane proteins including CD44v6, CD151, EGF R, Fas, Integrin α6/β4, Plexins B1, 2, 3, and MSP R/Ron (12-19). Ligation of one complex component triggers activation of the other, followed by cooperative signaling effects (12-19). Formation of some of these heteromeric complexes is a requirement for epithelial cell morphogenesis and tumor cell invasion (12, 16, 17). Paracrine induction of epithelial cell scattering and branching tubulogenesis results from the stimulation of HGF R on undifferentiated epithelium by HGF released from neighboring mesenchymal cells (20). Genetic polymorphisms, chromosomal translocation, overexpression, and additional splicing and proteolytic cleavage of HGF R have been described in a wide range of cancers (1). Within the ECD, human HGF R shares 86%-88% aa sequence identity with canine, mouse, and rat HGF R.

References:

1. Birchmeier, C. *et al.* (2003) Nat. Rev. Mol. Cell Biol. **4**:915.
2. Corso, S. *et al.* (2005) Trends Mol. Med. **11**:284.
3. Gherardi, E. *et al.* (2003) Proc. Natl. Acad. Sci. **100**:12039.
4. Park, M. *et al.* (1987) Proc. Natl. Acad. Sci. **84**:6379.
5. Crepaldi, T. *et al.* (1994) J. Biol. Chem. **269**:1750.
6. Prat, M. *et al.* (1991) Mol. Cell. Biol. **12**:5954.
7. Rodrigues, G.A. *et al.* (1991) Mol. Cell. Biol. **11**:2962.
8. Kong-Beltran, M. *et al.* (2004) Cancer Cell **6**:75.
9. Naldini, L. *et al.* (1991) Mol. Cell. Biol. **11**:1793.
10. Ponzetto, C. *et al.* (1994) Cell **77**:261.
11. Jeffers, M. *et al.* (1997) Mol. Cell. Biol. **17**:799.
12. Orian-Rousseau, V. *et al.* (2002) Genes Dev. **16**:3074.
13. Klosek, S.K. *et al.* (2005) Biochem. Biophys. Res. Commun. **336**:408.
14. Jo, M. *et al.* (2000) J. Biol. Chem. **275**:8806.
15. Wang, X. *et al.* (2002) Mol. Cell **9**:411.
16. Trusolino, L. *et al.* (2001) Cell **107**:643.
17. Giordano, S. *et al.* (2002) Nat. Cell Biol. **4**:720.
18. Conrotto, P. *et al.* (2004) Oncogene **23**:5131.
19. Follenzi, A. *et al.* (2000) Oncogene **19**:3041.
20. Sonnenberg, E. *et al.* (1993) J. Cell Biol. **123**:223.

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