# 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.<br>*Small pack size (-SP) is supplied as a 0.2 μm filtered solution in PBS. |

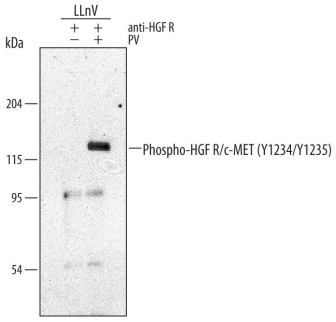
## APPLICATIONS

**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 |

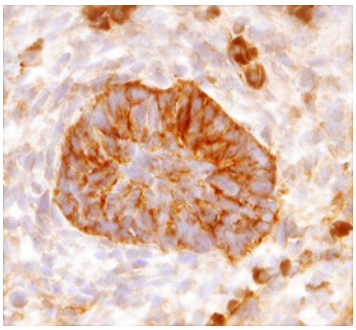
## DATA

### Western Blot



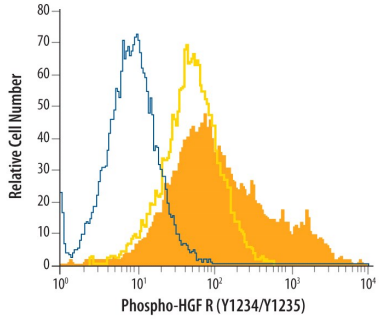

**Detection of Human Phospho-HGF R/c-MET (Y1234/Y1235) by Western Blot.** Western blot shows Goat Anti-Human HGF R/c-MET Antigen Affinity-purified Polyclonal Antibody (Catalog # AF276) immunoprecipitate of MDA-MB-468 human breast cancer cell line untreated (-) or treated (+) with 100 µM pervanadate (PV) 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



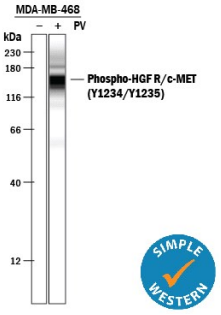
**HGF R/c-MET in Mouse Embryo.** HGF R/c-MET was detected in immersion fixed frozen sections of mouse embryo (13 d.p.c.) using Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480) at 15 µg/mL overnight at 4 °C. Tissue was stained using 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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## 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.<br>*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.**<br>● 12 months from date of receipt, -20 to -70 °C as supplied.<br>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.<br>● 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:**

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