

**DESCRIPTION**

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

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	0.5 µg/mL	See Below
<b>Immunohistochemistry</b>	5-15 µg/mL	See Below
<b>Intracellular Staining by Flow Cytometry</b>	2.5 µg/10 <sup>6</sup> cells	See Below
<b>Simple Western</b>	5 µg/mL	See Below
<b>CyTOF-reported</b>	Brodie, T.M. <i>et al.</i> (2018) <i>Cytometry Part A</i> . <b>93</b> : 406. 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.

**PREPARATION AND STORAGE**

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

**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  $\alpha$  chain and a 145 kDa transmembrane  $\beta$  chain (1, 2). The extracellular domain (ECD) contains a seven bladed  $\beta$ -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  $\alpha$  and  $\beta$  chains (5-7). The sema domain, which is formed by both the  $\alpha$  and  $\beta$  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  $\alpha 6/\beta 4$ , 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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