Human/Mouse Phospho-HGFR/c-MET (Y1349) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF3950

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse HGF R/c-MET when phosphorylated at Y1349.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human HGF R Y1349 site
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 either lyophilized or as a 0.2 μm filtered solution in PBS.

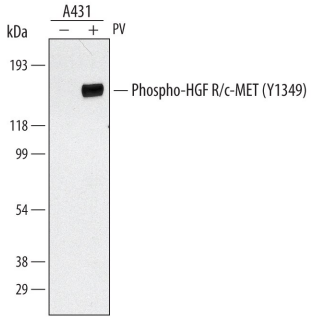
APPLICATIONS

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.*

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below

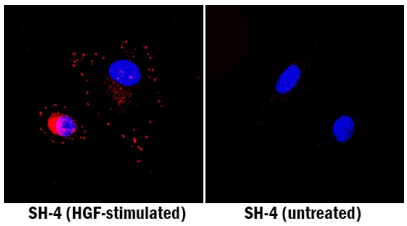
DATA

Western Blot



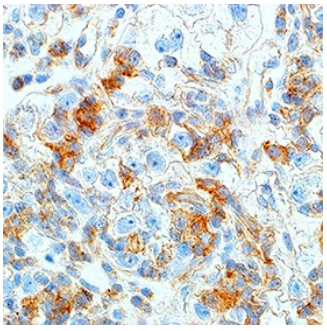
Detection of Human Phospho-HGF R/c-MET (Y1349) by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma cell line untreated (-) or treated (+) with 100 µM pervanadate (PV) for 10 minutes. PVDF membrane was probed with 0.5 µg/mL of Rabbit Anti-Human Phospho-HGF R/c-MET (Y1349) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3950), followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band was detected for Phospho-HGF R/c-MET (Y1349) at approximately 145 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry



HGF R/c-MET in SH-4 Human Cell Line. HGF R/c-MET was detected in immersion fixed SH-4 human melanoma cell line stimulated with HGF (left panel; positive staining) and non-stimulated (right panel; negative staining) using Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1349) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3950) at 15 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



HGF R/c-MET in Human Renal Cell Carcinoma Tissue. HGF R/c-MET was detected in immersion fixed paraffin-embedded sections of human renal cell carcinoma tissue using Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1349) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3950) at 15 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Rabbit IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC003). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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Human/Mouse Phospho-HGFR/c-MET (Y1349) Antibody

Antigen Affinity-purified Polyclonal Rabbit IgG

Catalog Number: AF3950

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:

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