Human HGF R/c-MET Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF276

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human HGF R/c-MET in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 30% cross-reactivity with recombinant mouse HGF R is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human HGF R/c-MET Glu25-Thr932 Accession # P08581
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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.

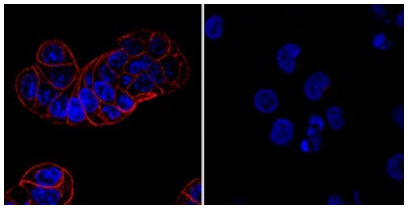
APPLICATIONS

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	0.1 µg/mL	Recombinant Human HGF R/c-MET Fc Chimera (Catalog # 358-MT)
Flow Cytometry	2.5 µg/10^6 cells	MDA-MB-231 human breast cancer cell line
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 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.	
Knockout Validated	HGF R/c-MET is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in HGF R/c-MET knockout HeLa cell line.	
Blockade of Receptor-ligand Interaction	In a functional ELISA, 0.5-2 µg/mL of this antibody will block 50% of the binding of 5 ng/mL of Recombinant Human HGF (Catalog # 294-HGN) to immobilized Recombinant Human HGF R/c-MET Fc Chimera (Catalog # 358-MT) coated at 1 µg/mL (100 µL/well). At 10 µg/mL, this antibody will block >90% of the binding.	

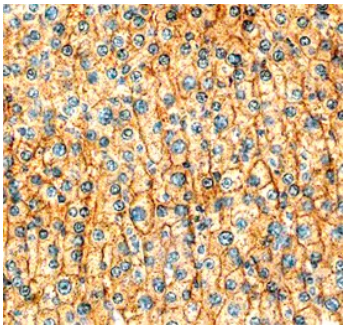
DATA

Immunocytochemistry



HGF R/c-MET in HT-29 and U937 Human Cell Line. HGF R/c-MET was detected in immersion fixed HT-29 human colon adenocarcinoma cell line (positive control, left panel) and U937 human histiocytic lymphoma cell line (negative control, right panel) using Goat Anti-Human HGF R/c-MET Antigen Affinity-purified Polyclonal Antibody (Catalog # AF276) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to plasma membrane. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry



HGF R/c-MET in Human Liver. HGF R/c-MET was detected in immersion fixed paraffin-embedded sections of human liver using Goat Anti-Human HGF R/c-MET Antigen Affinity-purified Polyclonal Antibody (Catalog # AF276) at 10 µg/mL overnight at 4 °C. 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 the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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Antigen Affinity-purified Polyclonal Goat IgG

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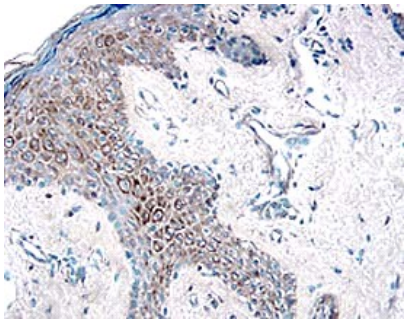
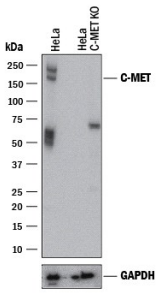
Immunohistochemistry



HGF R/c-MET in Human Skin. HGF R/c-MET was detected in immersion fixed paraffin-embedded sections of human skin using 15 µg/mL Goat Anti-Human HGF R/c-MET Antigen Affinity-purified Polyclonal Antibody (Catalog # AF276) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Knockout Validated



Western Blot Shows Human HGF R/c-MET Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and HGF R/c-Met knockout HeLa cell line (KO). PVDF membrane was probed with 1 µg/mL of Goat Anti-Human HGF R/c-MET Antigen Affinity-purified Polyclonal Antibody (Catalog # AF276) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for HGF R/c-MET at approximately 150-200 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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 non-covalent 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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