Human/Mouse Phospho-HGFR/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 either lyophilized or as a 0.2 µm filtered solution in PBS.

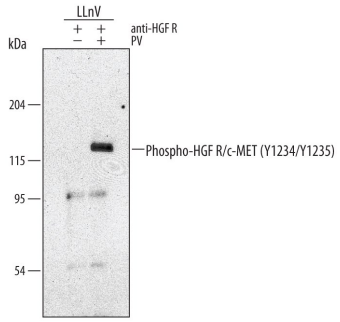
APPLICATIONS

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
Intracellular Staining by Flow Cytometry	2.5 µg/10^6 cells	See Below
Simple Western	5 µg/mL	See Below
CyTOF-reported	Brodie, T.M. *et al.* (2018) Cytometry Part A. **93**: 406. Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	

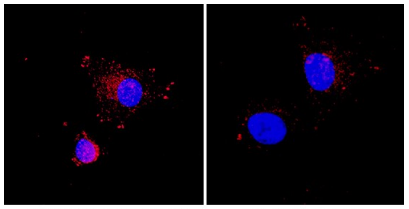
DATA

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.

Immunocytochemistry



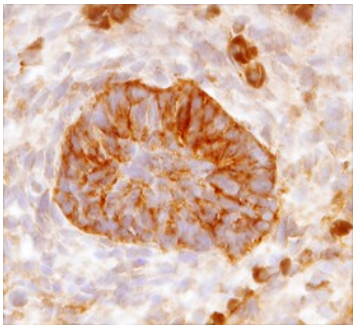
HGF R/c-MET in SH-4 Human Cell Line. HGF R/c-MET was detected in immersion fixed SH-4 human melanoma cell line stimulated with HGF (left panel; positive staining) and non-stimulated (right panel; negative staining) using Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480) 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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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

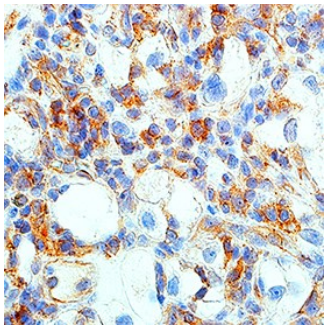
Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF2480

Immunohistochemistry



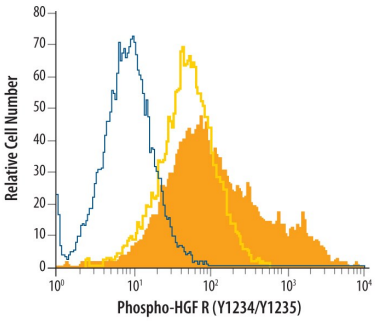
HGF R/c-MET in Mouse Embryo. HGF R/c-MET was detected in immersion fixed frozen sections of mouse embryo (13 d.p.c.) using Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480) at 15 µg/mL overnight at 4 °C. Tissue was stained using 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.

Immunohistochemistry



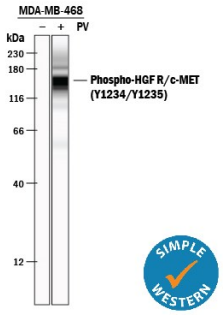
HGF R/c-MET in Human Renal Cell Carcinoma Tissue. HGF R/c-MET was detected in immersion fixed paraffin-embedded sections of human renal cell carcinoma tissue using Rabbit Anti-Human/Mouse Phospho-HGF R/c-MET (Y1234/Y1235) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2480) at 15 µg/mL for 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.

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

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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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

Antigen Affinity-purified Polyclonal Rabbit IgG
Catalog Number: AF2480

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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449