

### Certificate of Analysis

**Product:** Affinity Purified Anti-c-Met Proto-Oncogene Tyrosine Kinase pY1349 pY1356 [Rabbit] TRIAL SIZE

**Code:** 600-401-989S

**Lot #:** 20594

**Size:** 25  $\mu$ L

**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 1.47 mg/ml (by UV absorbance at 280 nm)

**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

**Storage Conditions for Trial Size:** Store vial at  $-20^{\circ}$  C or below prior to opening. This vial contains a relatively low volume of reagent (25  $\mu$ l). To minimize loss of volume dilute 1:10 by adding 225  $\mu$ l of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at  $-20^{\circ}$  C or below after dilution. Avoid cycles of freezing and thawing. Expiration date is three (3) months from date of opening.

**Background Information:** C-Met is the receptor for hepatocyte growth factor (also known as scatter factor, HGF/SF), and belongs to the tyrosine kinase superfamily. Interaction of c-Met with HGF results in autophosphorylation of c-Met at multiple tyrosines. Phosphorylation of Y1234/1235 in the c-Met kinase domain is critical to kinase activation. When phosphorylated, Y1349 and Y1356, along with surrounding amino acids, form a unique bidentate docking site for substrates such as Gab1, Grb2, phosphatidylinositol 3-kinase (PI3K) and others. C-Met mainly uses the Gab1 scaffolding adaptor in its initial step of signal transmission. Well-characterized downstream signalling pathways that are activated by c-Met include the ERK/MAPK, PI3K–Akt/PKB, Crk–Rap and Rac–Pak pathways, resulting in proliferation and increased cell survival as well as complex motogenic and morphogenic cellular responses. Gene targeting in mice has allowed elucidation of some of the developmental roles of c-Met and HGF/SF, such as their essential function in the formation of skeletal muscle, placenta and liver. Many types of cancer exhibit sustained c-Met stimulation, over-expression, or mutation, including carcinomas of the colon, breast, ovary, lung, liver, prostate, thyroid and kidney, as well as melanomas and sarcomas. In addition to cancer studies, other research areas in which c-Met is under investigation include organogenesis, organ regeneration, angiogenesis and surgical wound healing.

HGF - +



**Figure.** Western blot using Rockland's affinity purified anti-c-Met pY1349pY1356 antibody shows detection of phosphorylated c-Met. Human mammary B5/589 epithelial cells were serum-deprived and treated with or without HGF. Cell lysates were immunoprecipitated with the anti-c-Met antibody, resolved by SDS-PAGE, transferred to PVDF membrane, and probed with anti-c-Met pY1349pY1356. *Personal communication, D. Bottaro and T. Ito, NCI, Bethesda, MD*

**Application Note(s):** This affinity purified antibody has been tested for use in ELISA and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 150 kDa in size corresponding to phosphorylated c-Met protein by western blotting in the appropriate cell lysate or extract. This phospho-specific polyclonal antibody reacts with human c-Met pY1349pY1356 and shows minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide.

**Recommended Dilutions:**

ELISA	1:450,000
WESTERN BLOT	1:500 - 1:2,000
IMMUNOHISTOCHEMISTRY	User Optimized
OTHER APPLICATIONS	User Optimized

**Purity and Specificity:** This product was affinity purified from monospecific antiserum by immunoaffinity chromatography using dual-phospho-peptide coupled to agarose beads followed by cross-adsorption against nonphospho-peptide. This antibody is specific for human c-Met protein phosphorylated at Y1349 and Y1356. A BLAST analysis was used to suggest cross-reactivity with c-Met from human, mouse and rat based on 100% homology with the immunizing sequence. Cross-reactivity with c-Met from other sources has not been determined.

**Immunogen:** This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to residues surrounding Y1349 and Y1356 of human c-Met protein.

**Relevant Links:** NCBI [NP\\_000236](#) Swiss-Prot [P08581](#)

#### Related Products:

<a href="#">#611-703-127</a>	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
<a href="#">#611-132-122</a>	IRDye® 800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
<a href="#">#611-144-122</a>	DyLight™680 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
<a href="#">#B501-0500</a>	BLOTTO (500 g)
<a href="#">#BSA-30</a>	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
<a href="#">#B304</a>	NORMAL GOAT SERUM (NGS) (10 ml)
<a href="#">#KIA-003</a>	<b>MaxTag™</b> Anti-RABBIT IgG Kit for Immunoblotting
<a href="#">#MB-070</a>	Blocking Buffer for Fluorescent Western Blotting

#### General References:

Birchmeier, C., Birchmeier, W., Gherardi, E. and Van de Woude, G.F. (2003) Met, metastasis, motility and more. *Nat. Rev. Mol. Cell Bio.* **4**:915-925.

Fan, S., *et al.* (2001) The multisubstrate adapter Gab1 regulates hepatocyte growth factor (scatter factor)-c-Met signaling for cell survival and DNA repair. *Mol. Cell Biol.* **21**:4968-84.

Longati, P., *et al.* (2001) Receptor tyrosine kinases as therapeutic targets: the model of the MET oncogene. *Curr. Drug Targets* **2**:41-55.

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.