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Product Information

Anti-phospho-c-Met [pTyr^{1230}/pTyr^{1234}/pTyr^{1235}]
Developed in Rabbit, Affinity Isolated Antibody

Catalog Number **C7240**

Product Description

Anti-phospho-c-Met [pTyr^{1230}/pTyr^{1234}/pTyr^{1235}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of c-Met that contains Tyr^{1230}, Tyr^{1234}, and Tyr^{1235} as immunogen. The sequence is conserved in human, mouse, and rat. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated c-Met peptide. The antibody specifically recognizes c-Met phosphorylated on Tyr^{1230}, Tyr^{1234}, and Tyr^{1235}.

The antibody detects human and mouse c-Met. Rat (100% homologous) has not been tested, but is expected to cross-react. It has been used in immunoblotting applications.
Note: There are three isoforms of c-Met, two of which are recognized by this antibody.

Binding of scatter factor (SF)/hepatocyte growth factor (HGF) to the c-Met receptor tyrosine kinase (RTK) triggers receptor dimerization and phosphorylation on multiple residues within the juxtamembrane, catalytic core, and cytoplasmic tail domains, thereby, regulating receptor internalization, catalytic activity, and multisubstrate docking. c-Met contains three tyrosines (Tyr-xx-x-Tyr-Tyr motif) within the activation loop of the catalytic domain. This is also seen with the insulin receptor, insulin-like growth factor receptor (IGF1R), and nerve growth factor (NGF) receptors/Trks, for which phosphorylation of all three tyrosines is required for full activation.

Phosphorylation of Tyr^{1234} and Tyr^{1235} of c-Met (and the related family member, RON) has been shown to be important in receptor activation. Activation of the c-Met receptor results in binding and/or phosphorylation of many intracellular signaling proteins including multiple adaptor proteins (e.g., Grb2, Shc, Cbl, Crk, cortactin, paxillin, and GAB1) and a variety of other signal transducers (e.g., PI 3-kinase, FAK, Src, ERK1&2, JNK1&2, PLCα, and STAT3). This phosphospecific antibody does not distinguish between the dually [pTyr^{1234}/pTyr^{1235}] and triply [pTyr^{1230}/pTyr^{1234}/pTyr^{1235}] phosphorylated forms of c-Met, both of which are likely to represent activated forms of this receptor.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free), and 0.05% sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at –20 °C. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working concentration of 0.1–1.0 μg/mL is determined by immunoblotting using 293T kidney cells transiently transfected with human c-Met and stimulated with HGF; mouse myeloma (SP-1) cells stimulated with HGF and A431 cells stimulated with EGF.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

Results

Peptide Competition

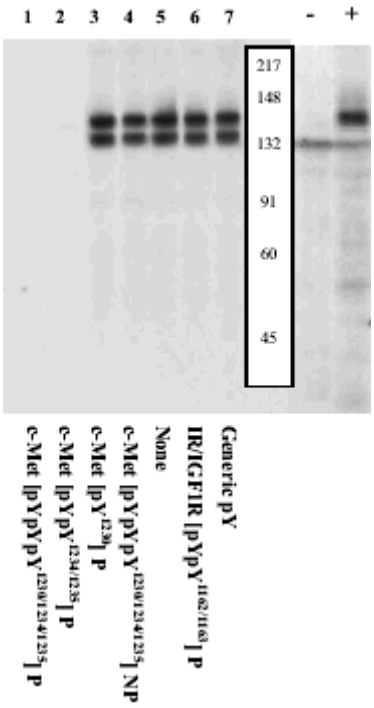
1. Extracts prepared from 293T cells transiently transfected with human c-Met were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
2. Membranes, were incubated with a 5% BSA-TBST buffer overnight at 2–8 °C, in order to block non-specific sites.
3. Subsequently the membranes were incubated as follows:

   Lane 1 – immunogen
   Lane 2 – a phosphorylated peptide corresponding to c-Met [pTyr^{1234}/pTyr^{1235}]
   Lane 3 – a phosphorylated peptide corresponding to c-Met [pTyr^{1230}]
   Lane 4 – the non-phosphopeptide corresponding to the immunogen
   Lane 5 – no peptide
   Lane 6 – a phosphorylated peptide corresponding to IR/IGF1R [pTyr^{1162}/pTyr^{1163}]
   Lane 7 – peptide containing generic phosphotyrosine

4. All lanes were incubated with 0.50 µg/mL c-Met [pTyr^{1230}/pTyr^{1234}/pTyr^{1235}] antibody.
5. After washing, membranes were incubated with goat F(ab′)$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

Figure 1.

Peptide competition



The data show that only the di and tri-phosphopeptides, corresponding to the c-Met [pTyr^{1230}/pTyr^{1234}/pTyr^{1235}] site, block the antibody signal, demonstrating the specificity of the c-Met [pTyr^{1230}/pTyr^{1234}/pTyr^{1235}] antibody for the activated forms of the c-Met receptor.

References

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