

## Product Information

### Anti-phospho-c-Met [pTyr<sup>1349</sup>]

produced in rabbit, affinity isolated antibody

Catalog Number **C6990**

#### Product Description

Anti-phospho-c-Met [pTyr<sup>1349</sup>] is produced in rabbit using a synthetic phosphorylated peptide derived from the region of c-Met that contains tyrosine 1349 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 tyrosine 1349.

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

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 tyrosine 1234 and 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, PLC- $\gamma$ , and STAT3). Tyrosine 1349 of c-Met is one of the primary docking sites for PI 3-kinase and GAB1.

The phosphorylation of tyrosines 1349 and 1356 serves as a priming mechanism for protein binding.

#### Reagent

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

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Storage/Stability

Store at -70 °C. Upon initial thawing, freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. 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 amount of supplied reagent is sufficient for 10 blots.

Immunoblotting: a recommended starting working dilution is 1:1000

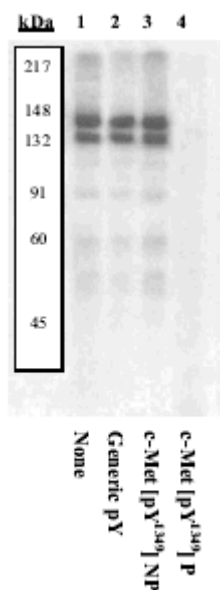
**Note:** In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

#### 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 4 °C, in order to block non-specific sites.
3. Subsequently the membranes were incubated as follows:
  - a. Lane 1 no peptide
  - b. Lane 2 peptide containing generic phosphotyrosine
  - c. Lane 3 the non-phosphopeptide corresponding to the immunogen
  - d. Lane 4 immunogen
4. All lanes were incubated with 0.50 µg/mL c-Met [pTyr<sup>1349</sup>] antibody
5. After washing, membranes were incubated with goat F(ab')<sub>2</sub> anti-rabbit IgG alkaline phosphatase and signals were detected.



**Figure 1: Peptide competition**

The data in Figure 1 show that only peptide corresponding to the c-Met [pTyr<sup>1349</sup>] site blocks the antibody signal, demonstrating the specificity of the c-Met [pTyr<sup>1349</sup>] antibody.

## References

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