Anti-Met (c-Met) antibody [EP1454Y] - N-terminal
ab51067

Product name
Anti-Met (c-Met) antibody [EP1454Y] - N-terminal

Description
Rabbit monoclonal [EP1454Y] to Met (c-Met) - N-terminal

Host species
Rabbit

Tested applications
Suitable for: WB, IHC-P, ICC/IF, Flow Cyt

Species reactivity
Reacts with: Mouse, Rat, Human

Immunogen
Synthetic peptide within Human Met (c-Met) aa 1-100 (N terminal). The exact sequence is proprietary.
Database link: P08581
(Peptide available as ab167073)

Positive control

General notes
A trial size is available to purchase for this antibody.
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a recombinant rabbit monoclonal antibody.

Properties
Form
Liquid

Storage instructions
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: PBS, 50% Glycerol, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EP1454Y

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab51067 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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</thead>
<tbody>
<tr>
<td>WB</td>
<td>1/1000 - 1/10000. Detects a band of approximately 160 kDa (predicted molecular weight: 156 kDa). Can be blocked with Human Met (c-Met) peptide (ab167073).</td>
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<tr>
<td>IHC-P</td>
<td>1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.</td>
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<tr>
<td>ICC/IF</td>
<td>1/100 - 1/250.</td>
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<tr>
<td>Flow Cyt</td>
<td>1/100 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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</table>

Target

Function
Receptor for hepatocyte growth factor and scatter factor. Has a tyrosine-protein kinase activity. Functions in cell proliferation, scattering, morphogenesis and survival.

Involvement in disease
Note=Activation of MET after rearrangement with the TPR gene produces an oncogenic protein. Note=Defects in MET may be associated with gastric cancer. Note=Defects in MET are a cause of hepatocellular carcinoma (HCC) [MIM:114550]. Note=Defects in MET are a cause of renal cell carcinoma papillary (RCCP) [MIM:605074]. It is a subtype of renal cell carcinoma tending to show a tubulo-papillary architecture formed by numerous, irregular, finger-like projections of connective tissue. Renal cell carcinoma is a heterogeneous group of sporadic or hereditary carcinoma derived from cells of the proximal renal tubular epithelium. It is subclassified into common renal cell carcinoma (clear cell, non-papillary carcinoma), papillary renal cell carcinoma, chromophobe renal cell carcinoma, collecting duct carcinoma with medullary carcinoma of the kidney, and unclassified renal cell carcinoma. Note=A common allele in the promoter region of the MET shows genetic association with susceptibility to autism in some families. Functional assays indicate a decrease in MET promoter activity and altered binding of specific transcription factor complexes. Note=MET activating mutations may be involved in the development of a highly malignant, metastatic syndrome known as cancer of unknown primary origin (CUP) or primary occult malignancy. Systemic neoplastic spread is generally a late event in cancer progression. However, in some instances, distant dissemination arises at a very early stage, so that metastases reach clinical relevance before primary lesions. Sometimes, the primary lesions cannot be identified in spite of the progresses in the diagnosis of malignancies.
Sequence similarities
Belongs to the protein kinase superfamily. Tyr protein kinase family.
Contains 3 IPT/TIG domains.
Contains 1 protein kinase domain.
Contains 1 Sema domain.

Domain
The kinase domain is involved in SPSB1 binding.

Post-translational modifications
Dephosphorylated by PTPRJ at Tyr-1349 and Tyr-1365.

Cellular localization
Membrane.

Images

Lane 1: Wild-type HAP1 cell lysate (40 µg)
Lane 2: Met (c-Met) knockout HAP1 cell lysate (40 µg)
Lane 3: HepG2 cell lysate (40µg) (40 µg)
Lane 4: HEK-293 cel lysate (40µg) (40 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab51067 observed at 240 kDa. Red - loading control, ab18058, observed at 124 kDa.

This western blot image is a comparison between ab51067 and a competitor's top cited rabbit polyclonal antibody.

ab51067 staining Met (c-Met) in human breast tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with formaldehyde, permeabilized with 0.05% Tween-20 and blocked for 30 minutes at 22°C; antigen retrieval was by heat mediation in antigen retrieval buffer (100X citrate buffer pH 6.0) (ab94674). Samples were incubated with the primary antibody (1/100) for 14 hours at 4°C. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/300) was used as the secondary antibody.
Immunohistochemical staining of paraffin embedded human bladder carcinoma with purified ab51067 at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counterstained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control (inset).

Immunohistochemical staining of paraffin embedded human clear cell kidney carcinoma with purified ab51067 at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (ab97051) at 1/500. The sample is counterstained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.

PBS was used instead of the primary antibody as the negative control (inset).
All lanes: Anti-Met (c-Met) antibody [EP1454Y] - N-terminal (ab51067) at 1/1000 dilution

Lane 1: Mouse thymus tissue lysate
Lane 2: Rat thymus tissue lysate
Lane 3: Mouse lung tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 156 kDa
**Observed band size:** 240 kDa

*why is the actual band size different from the predicted?*

Lanes 1 - 3: Merged signal (red and green). Green - ab51067 observed at 240 kDa. Red - loading control, ab18058, observed at 130 kDa.

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab51067 and ab18058 (loading control) overnight at 4°C. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at a 1:10000 dilution for 1hr at room temperature and then imaged.
Western blot - Anti-Met (c-Met) antibody [EP1454Y] - N-terminal (ab51067)

**All lanes**: Anti-Met (c-Met) antibody [EP1454Y] - N-terminal (ab51067) at 1/2000 dilution (purified)

**Lane 1**: HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

**Lane 2**: HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate

Lysates/proteins at 10 µg per lane.

**Secondary**

**All lanes**: HRP goat anti-rabbit IgG (H+L) at 1/20000 dilution

**Predicted band size**: 156 kDa

**Observed band size**: 190 kDa

_why is the actual band size different from the predicted?_

Blocking/Dilution buffer: 5% NFDM/TBST.

Immunofluorescence staining of Jurkat (Human T cell leukemia cell line from peripheral blood) cells with purified ab51067 at a working dilution of 1/100, counterstained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (ab150077), used at a dilution of 1/1000. ab7291, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with ab150120 (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel.

The cells were fixed in 100% methanol and permeabilized using 0.1% Triton X 100.

The negative controls are shown in bottom middle and right hand panels - for **negative control 1**, purified ab51067 was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (ab150120) at a dilution of 1/500. For **negative control 2**, ab7291 (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (ab150077) at a dilution of 1/400.
Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells fixed in 4% PFA and stained with purified ab51067 at a dilution of 1 in 100 (red line). The secondary antibody used was FITC goat anti-rabbit at a dilution of 1 in 500. Rabbit monoclonal IgG was used as an isotype control (black line) and cells incubated in the absence of both primary and secondary antibody were used as a negative control (blue line).

Overlay histogram showing Jurkat (Human T cell leukemia cell line from peripheral blood) cells stained with unpurified ab51067 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab51067, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit Alexa Fluor® 488 (IgG, H+L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1μg/1x10⁶ cells) used under the same conditions. Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.
Lane 1: Wild-type HAP1 cell lysate (40 µg)
Lane 2: Met (c-Met) knockout HAP1 cell lysate (40 µg)
Lane 3: HepG2 cell lysate (40 µg) (40 µg)
Lane 4: HEK-293 cell lysate (40 µg) (40 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab51067 observed at 240 kDa. Red - loading control, ab18058, observed at 124 kDa.

ab51067 was shown to specifically recognize Met (c-Met) in wild-type HAP1 cells along with additional cross reactive bands. No bands were observed when Met (c-Met) knockout samples were used. Wild-type and Met (c-Met) knockout samples were subjected to SDS-PAGE. ab51067 and ab18058 (loading control to Vinculin) were diluted at 1/1000 and 1/10,000 dilution respectively and incubated overnight at 4C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10,000 dilution for 1hr at room temperature before imaging.

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