Product datasheet

Anti-MMP14 antibody [EP1264Y] ab51074

Product name: Anti-MMP14 antibody [EP1264Y]
Description: Rabbit monoclonal [EP1264Y] to MMP14
Host species: Rabbit
Tested applications: Suitable for: Flow Cyt, IHC-P, IP, WB, ICC/IF
Species reactivity: Reacts with: Mouse, Rat, Human
Predicted to work with: Cow
Immunogen: Synthetic peptide within Human MMP14 aa 150-250. The exact sequence is proprietary.
Database link: P50281
(Peptide available as ab185123)
General notes: A trial size is available to purchase for this antibody.
The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples.
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 buffer
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EP1264Y

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab51074 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tr>
<td>Flow Cyt</td>
<td>★★★★★</td>
<td>1/20.</td>
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<tr>
<td>IHC-P</td>
<td>★★★★★</td>
<td>1/100.</td>
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</table>
| IP          | ★★★★★     | 1/20.  
For unpurified use at 1/100. |
| WB          | ★★★★★     | 1/5000.  
1 Predicted molecular weight: 66 kDa. Can be blocked with MMP14 peptide (ab185123).  
For unpurified use at 1/2000. |
| ICC/IF      | ★★★★★     | Use at an assay dependent concentration. |

Target

Function
Seems to specifically activate progelatinase A. May thus trigger invasion by tumor cells by activating progelatinase A on the tumor cell surface. May be involved in actin cytoskeleton reorganization by cleaving PTK7.

Tissue specificity
Expressed in stromal cells of colon, breast, and head and neck. Expressed in lung tumors.

Sequence similarities
Belongs to the peptidase M10A family. Contains 4 hemopexin-like domains.

Domain
The conserved cysteine present in the cysteine-switch motif binds the catalytic zinc ion, thus inhibiting the enzyme. The dissociation of the cysteine from the zinc ion upon the activation-peptide release activates the enzyme.

Post-translational modifications
The precursor is cleaved by a furin endopeptidase.

Cellular localization
Membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images
Western blot - Anti-MMP14 antibody [EP1264Y] (ab51074)

All lanes: Anti-MMP14 antibody [EP1264Y] (ab51074) at 1/2000 dilution (unpurified)

Lane 1: Human fetal spleen tissue lysate
Lane 2: Human lung cancer tissue lysate
Lane 3: MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 66 kDa
Observed band size: 60,63 kDa
why is the actual band size different from the predicted?

Exposure time: 10 seconds

Blocking/Diluting buffer and concentration 5% NFDM/TBST
63 kDa: pro-form; 60 kDa: active form.

MCF7 is a MMP14 negative or weakly expressed cell line (PMID: 25977338 and PMID: 19208838).

Western blot - Anti-MMP14 antibody [EP1264Y] (ab51074)

All lanes: Anti-MMP14 antibody [EP1264Y] (ab51074) at 1/5000 dilution (purified)

Lane 1: Human fetal spleen lysate
Lane 2: Human lung cancer lysate
Lane 3: Mouse spleen lysate
Lane 4: Rat spleen lysate
Lane 5: Human esophagus lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 66 kDa
Observed band size: 66 kDa
Blocking and diluting buffer: 5% NFDM/TBST.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human endometrium carcinoma tissue sections labeling MMP14 with purified ab51074 at 1/100 dilution (1.7 μg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, PH9. Hematoxylin was used to counterstain. ab97051, a Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1/500 dilution.

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

ab51074 (unpurified) at 1/500 staining human kidney tissue sections by IHC-P.

The tissue was formaldehyde fixed and a heat mediated antigen retrieval step (in Tris/EDTA) was performed. The tissue was then blocked with serum and incubated with the primary antibody. A biotinylated donkey anti-rabbit IgG was used as the secondary.
Ab51074 staining MMP14 in HT-1080 (human fibrosarcoma epithelial cell) cells by ICC/IF (Immunocytochemistry/Immunofluorescence).

Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at 1/1000 dilution (0.2 μg/ml). An Alexa Fluor® 488 Goat anti-rabbit (ab150077) was used as the secondary antibody at 1/1000 dilution (2 μg/ml). Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594, ab195889) was used as the counterstain antibody at 1/200 dilution (2.5 μg/ml). DAPI was used as a nuclear counterstain. Confocal image showing cytoplasmic and weakly membranous staining in HT-1080 cell line.

**Negative control (bottom panels):** MCF7 PMID: 19208838.

ab51074 (purified) at 1/20 dilution (2 μg) immunoprecipitating MMP14 in A431 (human epidermoid carcinoma) whole cell lysate.

**Lane 1:** A431 whole cell lysate 10ug  
**Lane 2:** ab51074 + A431 whole cell lysate  
**Lane 3:** Rabbit monoclonal IgG (ab172730) instead of ab51074 in A431 whole cell lysate

For western blotting, ab131366 VeriBlot for IP (HRP) was used as the secondary antibody (1/1000).

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
Flow cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell, Left) / HT-1080 (Human fibrosarcoma epithelial cell, Right) cells labeling MMP14 with ab51074 at 1/200 dilution (0.1 μg) (red). Goat anti-rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/2000 dilution. Rabbit monoclonal IgG (ab172730) / black was used as the isotype control. Cells incubated with secondary antibody only (blue) was used as the unlabeled control. Gated on viable cells.

**Positive control (Right panel):** HT-1080 cells.

**Negative control (Left panel):** MCF7 cells.

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