

Product datasheet

Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free ab168726


KO VALIDATED

Recombinant

RabMAb

8 Images

Overview

Product name	Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EP1264Y] to MMP14 - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	Suitable for: ICC/IF, Flow Cyt (Intra), WB, IHC-P, IP
Species reactivity	Reacts with: Mouse, Rat, Human Predicted to work with: Cow 
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human endometrium carcinoma and kidney tissues. WB: Human fetal spleen, esophagus and lung cancer tissue lysates, mouse and rat spleen tissue lysates; A431 wild-type cell lysate and Caco-2 whole cell lysate. Flow Cyt (intra): HT-1080 cells. IP: A431 cell lysate. ICC/IF: HT-1080 cells and wild-type A431 cells.
General notes	<p>ab168726 is the carrier-free version of ab194242.</p> <p>Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.</p> <p>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.</p> <p>Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production

For more information [see here](#).

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMAb[®] patents](#).

Our **Low endotoxin, azide-free formats** have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1264Y
Isotype	IgG

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab168726 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 65 kDa. For unpurified use at 1/2000.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration. For unpurified use at 1/100.

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.

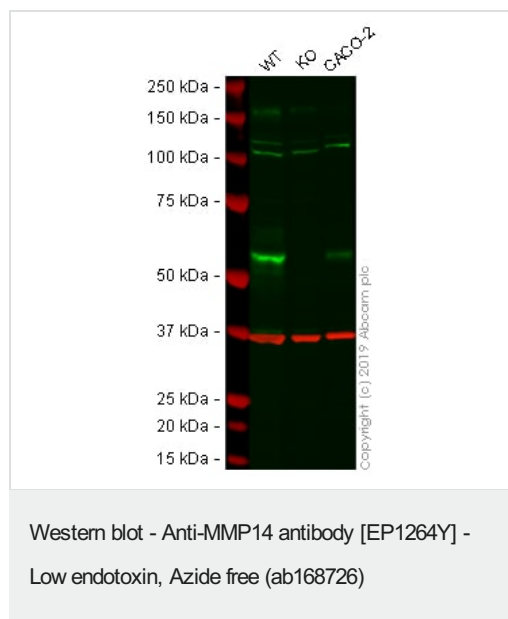
Post-translational modifications

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



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

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 2 : MMP14 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate

Lane 3 : Caco-2 (Human colorectal adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 65 kDa

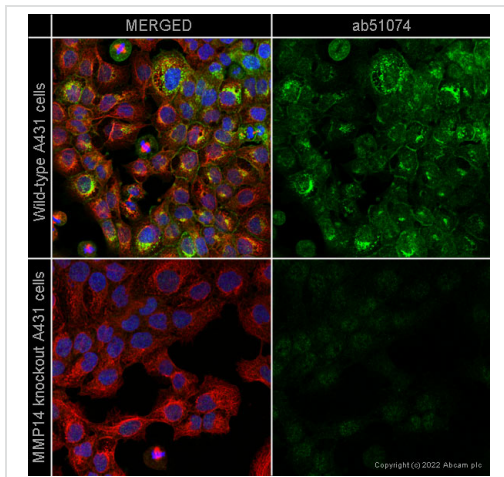
Observed band size: 54 kDa

This data was developed using the same antibody clone in a different buffer formulation ([ab51074](#)).

Lanes 1 - 3: Merged signal (red and green). Green - [ab51074](#) observed at 54 kDa. Red - loading control, [ab8245](#) (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

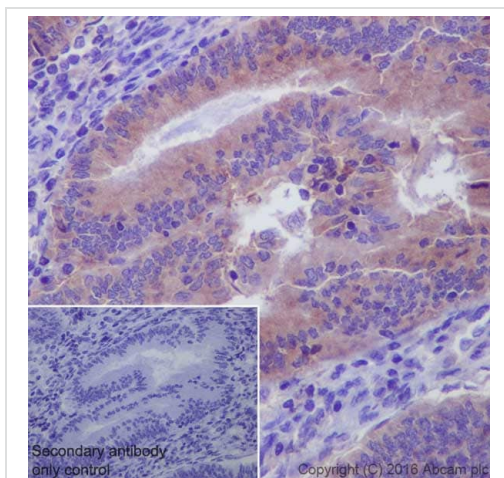
[ab51074](#) was shown to react with MMP14 in A431 wild-type cells in Western blot. Loss of signal was observed when MMP14 knockout sample was used. A431 wild-type and MMP14 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% Milk in TBS-T (0.1% Tween®) before incubation with [ab51074](#) and [ab8245](#) (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were

developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free ([ab168726](#))

This data was developed using the same antibody clone in a different buffer formulation ([ab51074](#)). [ab51074](#) staining MMP14 in wild-type A431 cells (top panel) and MMP14 knockout A431 cells (bottom panel) ([ab261890](#)). The cells were fixed with 4% paraformaldehyde (10 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with [ab51074](#) at 0.2µg/ml concentration and [ab7291](#) (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) ([ab150081](#)) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) ([ab150120](#)) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI. Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

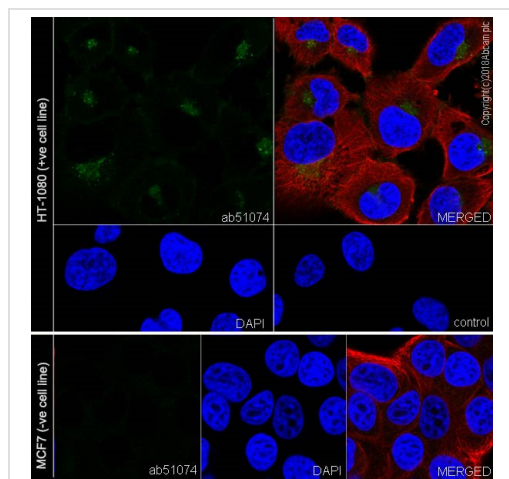


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free ([ab168726](#))

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab51074](#)).

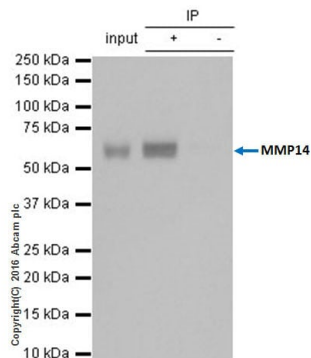


Immunocytochemistry/ Immunofluorescence - Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free (ab168726)

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.



Immunoprecipitation - Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free (ab168726)

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

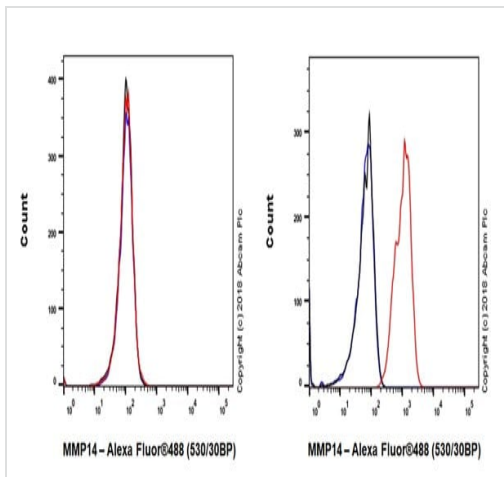
Lane 2: **ab51074** + A431 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab51074** in A431 whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution.

Blocking and diluting buffer and concentration: 5% NFDm/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51074**).

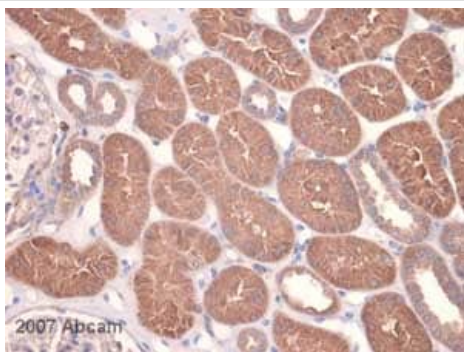


Flow Cytometry (Intracellular) - Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free (ab168726)

Intracellular 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP14 antibody [EP1264Y] - Low endotoxin, Azide free (ab168726)
This image is courtesy of an anonymous Abreview.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab51074**).

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

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Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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