


Anti-MMP14 antibody [EP1264Y] ab51074

敲除验证 重组 RabMAb

★★★★☆ 20 Abreviews 161 References 12 图像

概述

产品名称	Anti-MMP14抗体[EP1264Y]
描述	兔单克隆抗体[EP1264Y] to MMP14
宿主	Rabbit
经测试应用	适用于: IHC-P, IP, Flow Cyt (Intra), WB, ICC/IF
种属反应性	与反应: Mouse, Rat, Human 预测可用于: Cow 
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as ab185123)
阳性对照	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.
常规说明	The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- 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](#).

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20

	Preservative: 0.01% Sodium azide
	Constituents: PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EP1264Y
同种型	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab51074于以下的经测试应用

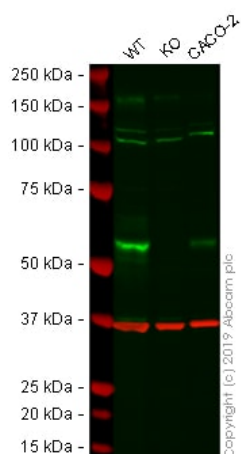
“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IHC-P	★★★★★ (4)	1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/20. For unpurified use at 1/100.
Flow Cyt (Intra)		1/200.
WB	★★★★★ (8)	1/5000. Predicted molecular weight: 66 kDa.Can be blocked with <u>MMP14 peptide (ab185123)</u> . For unpurified use at 1/2000.
ICC/IF	★★★★★ (6)	Use a concentration of 0.2 µg/ml.

靶标

功能	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.
组织特异性	Expressed in stromal cells of colon, breast, and head and neck. Expressed in lung tumors.
序列相似性	Belongs to the peptidase M10A family. Contains 4 hemopexin-like domains.
结构域	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.
翻译后修饰	The precursor is cleaved by a furin endopeptidase.
细胞定位	Membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

图片



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

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.

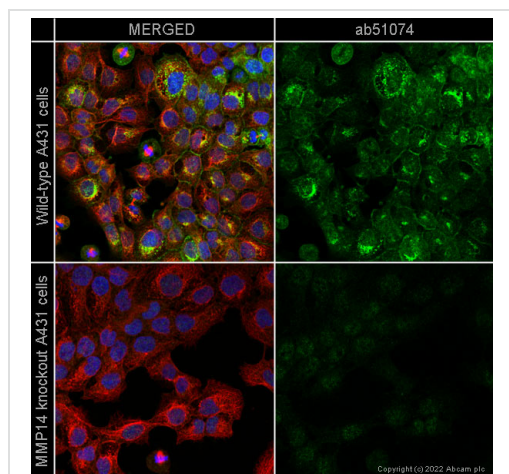
Performed under reducing conditions.

Predicted band size: 66 kDa

Observed band size: 54 kDa

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.

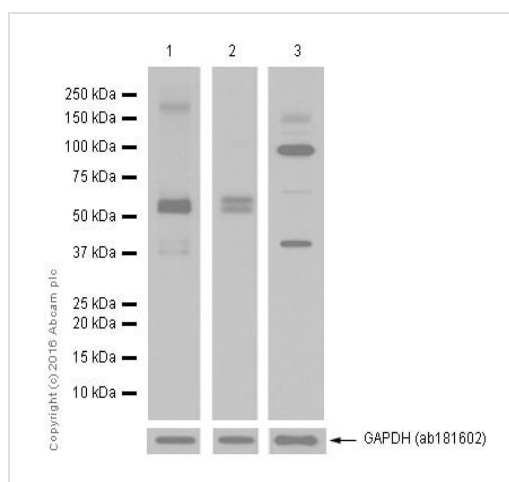
ab51074 was shown to react with MMP14 in A431 wild-type cells in Western blot. Loss of signal was observed when MMP14 knockout sample was used. A431 wild-type and MMP14 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% Milk in TBS-T (0.1% Tween®) before incubation with ab51074 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] (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).



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

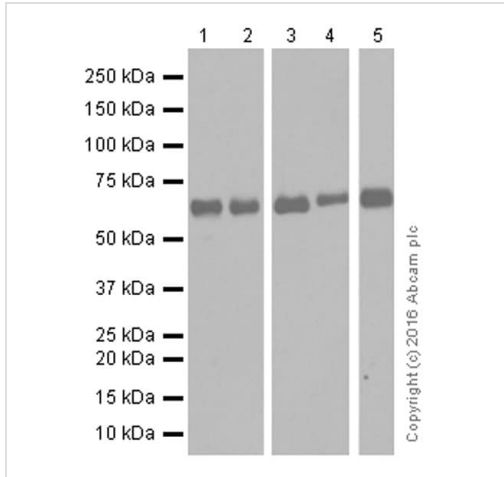
Observed band size: 60,63 kDa

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.

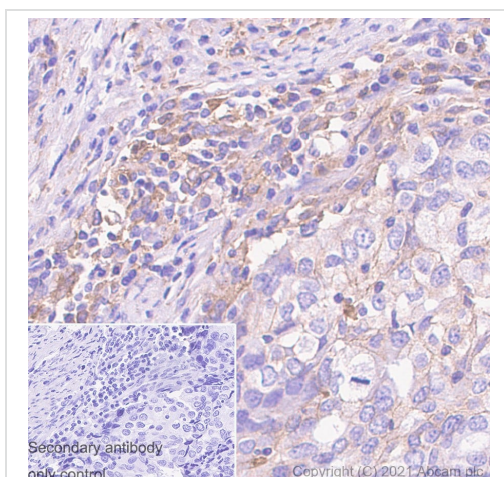
Secondary

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.

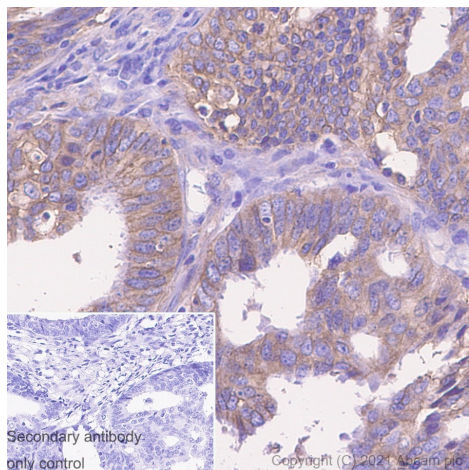


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP14 antibody [EP1264Y] (ab51074)

Immunohistochemical analysis of paraffin-embedded Human endometrial cancer labeling MMP14 with ab51074 at 1/500 (3.26 µg/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB

(ab209101). Positive staining on the human endometrial tumour stroma cells and weak staining on the tumour cells. The section was incubated with ab51074 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

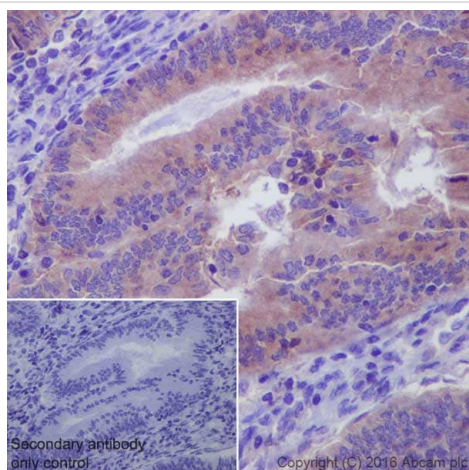
Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP14 antibody [EP1264Y] (ab51074)

Immunohistochemical analysis of paraffin-embedded Human endometrial cancer labeling MMP14 with ab51074 at 1/500 (3.26 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on human endometrial cancer. The section was incubated with ab51074 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

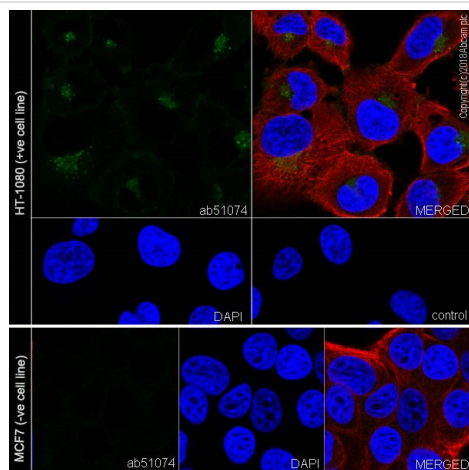
Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP14 antibody [EP1264Y] (ab51074)

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).

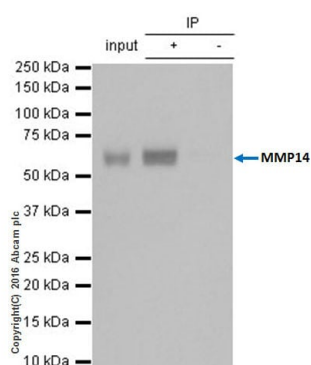


Immunocytochemistry/ Immunofluorescence - Anti-MMP14 antibody [EP1264Y] (ab51074)

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] (ab51074)

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

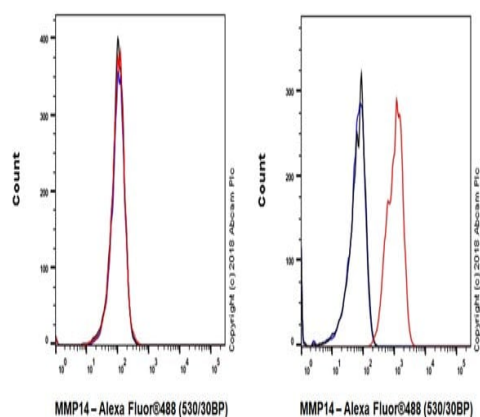
Lane 1: A431 whole cell lysate 10ug

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 for detection (1/1000).

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

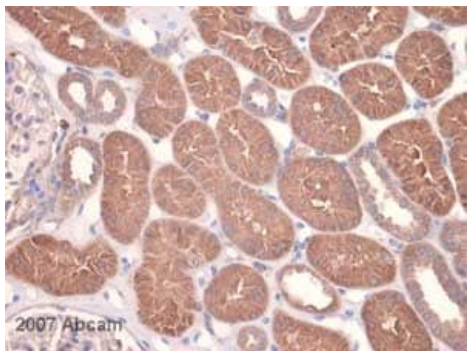


Flow Cytometry (Intracellular) - Anti-MMP14 antibody [EP1264Y] (ab51074)

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] (ab51074)

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.

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Recombinant technology



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Confirmed specificity



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Anti-MMP14 antibody [EP1264Y] (ab51074)

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