

Anti-MMP9 antibody [EP1254] ab76003

重组 RabMAb

★★★★★ **12 Abreviews** **451 References** **15 图像**

概述

产品名称	Anti-MMP9抗体[EP1254]
描述	兔单克隆抗体[EP1254] to MMP9
宿主	Rabbit
特异性	Based on our preliminary data, ab76003 detects no or weak band of interest in the untreated cell lines at the dilution of 1/200. Treatment increasing the expression of MMP-9 is recommended when using this antibody.
经测试应用	适用于: Flow Cyt (Intra), ICC/IF, WB, IHC-P
种属反应性	与反应: Rat, Human, Recombinant fragment
免疫原	Synthetic peptide within Human MMP9 aa 100-200. The exact sequence is proprietary. Database link: P14780
阳性对照	WB: U937, HL60 and TPA treated HT1080 cell lysates, human and rat lung, spleen and lymph node tissue lysate. IHC-P: Human gastric adenocarcinoma and spleen tissue. ICC/IF: U-2 OS and domoic acid-treated U87-MG cells. Flow Cyt (intra): A431 cells.
常规说明	<p>This antibody works better in 1% SDS Hot Lysates in WB. For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or <u>here (downloadable copy)</u>.</p> <p>Mouse: We have internal testing data to indicate this antibody reacts with this species in immunohistochemical and ELISA-based applications, but we were unable to detect a band with mouse lysates in western blot. Please contact us for more information.</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 <p>For more information <u>see here</u>.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>.</p>

性能

形式 Liquid

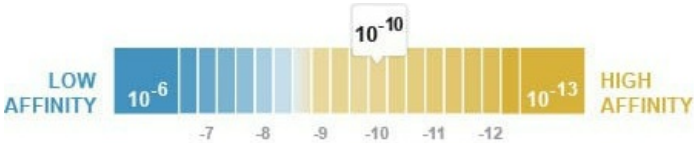
存放说明

Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

解离常数 (K_D)

K_D = 1.58 x 10⁻¹⁰ M



[Learn more about K_D](#)

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

EP1254

同种型

IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab76003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
Flow Cyt (Intra)		1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/250 - 1/500.
WB	★★★★★ (7)	1/1000 - 1/20000. Detects a band of approximately 92 kDa (predicted molecular weight: 78 kDa). Based on our preliminary data, ab76003 detects no or weak band of interest in the untreated cell lines at the dilution of 1/200. Treatment increasing the expression of MMP-9 is recommended when using this antibody.
IHC-P	★★★★★ (4)	1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See <u>IHC antigen retrieval protocols.</u> For unpurified use at 1/100 - 1/250.

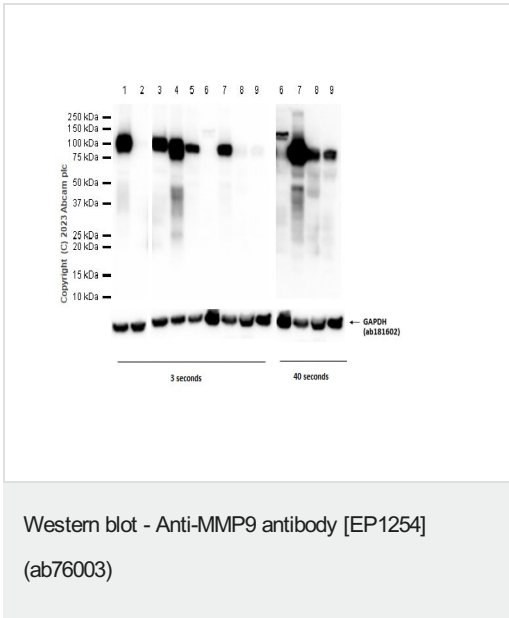
靶标

功能

May play an essential role in local proteolysis of the extracellular matrix and in leukocyte migration. Could play a role in bone osteoclastic resorption. Cleaves KiSS1 at a Gly-Leu bond. Cleaves type IV and type V collagen into large C-terminal three quarter fragments and shorter N-terminal one quarter fragments. Degrades fibronectin but not laminin or Pz-peptide.

组织特异性	Produced by normal alveolar macrophages and granulocytes.
疾病相关	Intervertebral disc disease Metaphyseal anadysplasia 2
序列相似性	Belongs to the peptidase M10A family. Contains 3 fibronectin type-II domains. Contains 4 hemopexin repeats.
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	Processing of the precursor yields different active forms of 64, 67 and 82 kDa. Sequentially processing by MMP3 yields the 82 kDa matrix metalloproteinase-9. N- and O-glycosylated.
细胞定位	Secreted, extracellular space, extracellular matrix.

图片



All lanes : Anti-MMP9 antibody [EP1254] (ab76003) at 1/1000 dilution

- Lane 1 :** Human lung tissue lysate
- Lane 2 :** Human brain tissue lysate
- Lane 3 :** Human spleen tissue lysate
- Lane 4 :** Human lymph node tissue lysate
- Lane 5 :** Rat lung tissue lysate
- Lane 6 :** Rat brain tissue lysate
- Lane 7 :** Rat spleen tissue lysate
- Lane 8 :** Rat kidney tissue lysate
- Lane 9 :** Rat lymph node tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 78 kDa

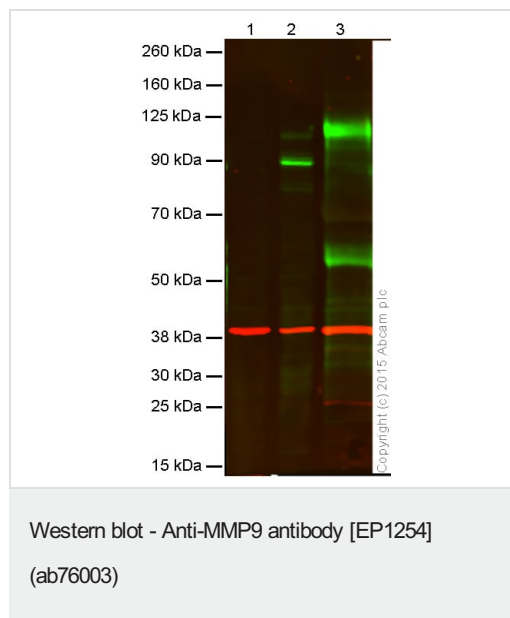
Observed band size: 84-92 kDa

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

Exposure time: 3 seconds; 40 seconds.

[ab181602](#) was used as loading control for GAPDH.

Although MMP9 has been studied in brain in some publications, ab76003 was unable to detect signal in normal brain tissue, this may be because MMP9 expression level is low in normal brain and would be increased in abnormal conditions like injury (PMID: 31198417).



All lanes : Anti-MMP9 antibody [EP1254] (ab76003) at 1.5 µg/ml

Lane 1 : Control U937 at 100 µg

Lane 2 : Stimulated U937 (24 hours with 10 ng x mL⁻¹ PMA ([ab120297](#)), 3 final hours with 3 ug x mL⁻¹ of Brefeldin ([ab120299](#))) at 100 µg

Lane 3 : Human tonsils at 20 µg

Secondary

All lanes : Goat anti-rabbit at 1/10000 dilution

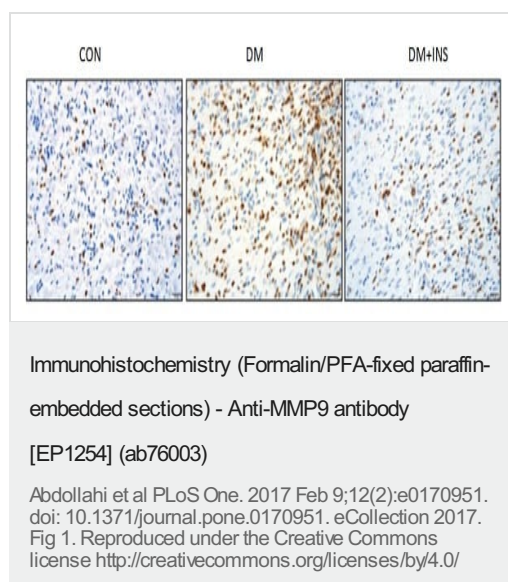
Predicted band size: 78 kDa

Observed band size: 89 kDa

Running buffer: MOPS.

Conditions: Denatured/reduced.

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with ab76003 (rabbit-anti MMP9; 1.5 ug/mL) and [ab8245](#) (loading control to GAPDH; 0.1 ug/mL) for 48 hours at 4°C. Before imaging, antibody binding was detected using infrared-labeled goat anti-rabbit (green) and goat anti-mouse (red) at 1:10,000 dilution for 1 hour at room temperature.

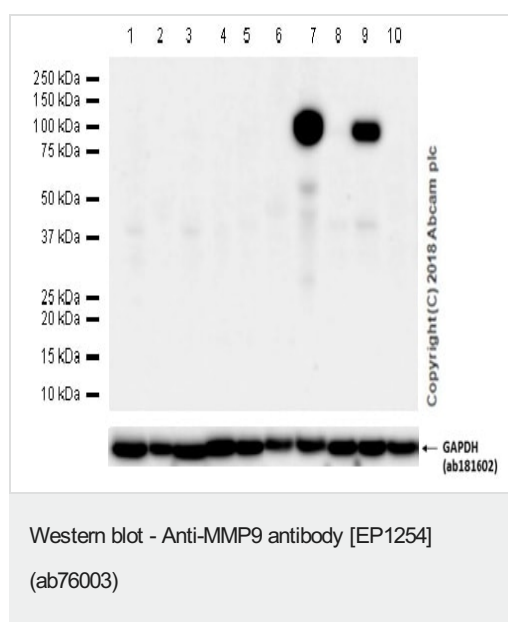


Representative images for skin wound tissue stained for MMP9.

The effect of diabetes on granulation tissue MMP-9 was also studied in a skin excisional wound model. For these studies, the rats were anaesthetized and the dorsum was prepared for wounding. Four full-thickness circular wounds (8mm²) were then created on the dorsum using a biopsy punch as previously described. Wound area was traced daily for determination of wound healing rate (calculated as change in wound area /day) and at day 6 post wounding the animals (n = 5-6/group) were euthanized and the skin containing the wound tissue was excised. Two wounds were snap frozen in liquid N₂ for later measurement of gene expression and the other wounds were divided in half and fixed in formalin (10%) for histological and immunohistological studies or frozen in OCT for immunofluorescence staining.

Results are from control (CON) diabetic (DM) and insulin treated DM (DM+INS) animals.

For full image please see paper.



All lanes : Anti-MMP9 antibody [EP1254] (ab76003) at 1/200 dilution (purified)

Lane 1 : LoVo (Human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : Huh7 (Human hepatocellular carcinoma epithelial cell) whole cell lysate

Lane 3 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 4 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 5 : Caco-2 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate

Lane 6 : A549 (Human lung carcinoma epithelial cell) serum starved overnight whole cell lysate

Lane 7 : A549 (Human lung carcinoma epithelial cell) serum starved overnight, then treated with 80nM TPA for 24 hours whole cell lysate

Lane 8 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) serum starved overnight whole cell lysate

Lane 9 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) serum starved overnight, then treated with 200nM TPA for 24 hours whole cell lysate

Lane 10 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 78 kDa

Observed band size: 84-82 kDa

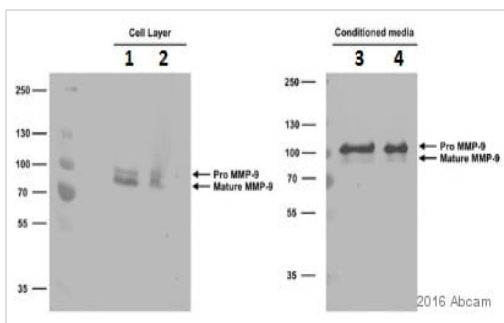
Exposure time: 60 seconds

Blocking and dilution buffer: 5% NFDM/TBST.

The expression of MMP-9 can be stimulated by various agents, such as inflammatory cytokine, growth factor, and 12-O-tetradecanoylphorbol-13-acetate (TPA) (PMID:21047770, 28969043).

Based on our preliminary data, ab76003 detects no or weak band of interest in the untreated cell lines at the dilution of 1/200.

Treatment increasing the expression of MMP-9 is recommended when using this antibody.



Western blot - Anti-MMP9 antibody [EP1254]
(ab76003)

Data courtesy of an anonymous AbReview

All lanes : Anti-MMP9 antibody [EP1254] (ab76003) at 1/5000 dilution

Lanes 1-2 : HTB94 (human chondrosarcoma cell line) cell lysate

Lanes 3-4 : HTB94 (human chondrosarcoma cell line) conditioned media

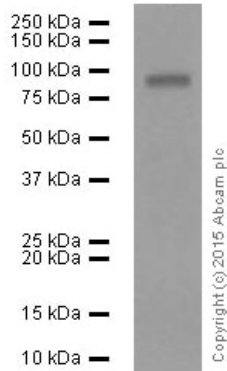
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat anti-rabbit IgG at 1/5000 dilution

Developed using the ECL technique.

Predicted band size: 78 kDa



Western blot - Anti-MMP9 antibody [EP1254]
(ab76003)

Anti-MMP9 antibody [EP1254] (ab76003) at 1/1000 dilution
(purified) + Rat kidney tissue lysate at 10 µg

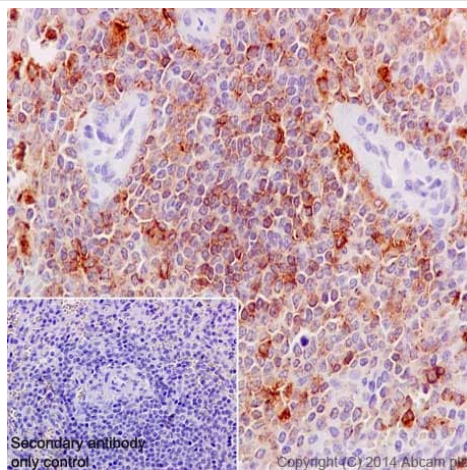
Secondary

Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 78 kDa

Observed band size: 84-92 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

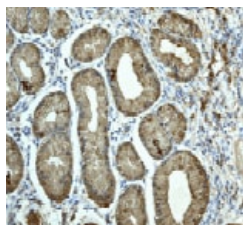


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody
[EP1254] (ab76003)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human spleen tissue labeling MMP9 with purified ab76003 at 1/1000. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. [ab97051](#), an HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500).

Negative control using PBS instead of primary antibody (inset).

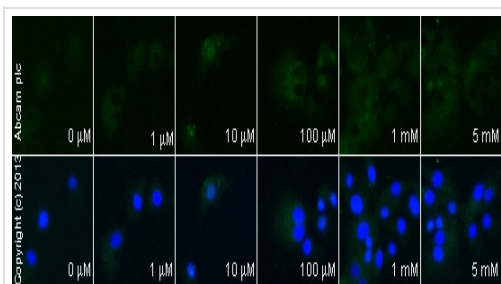
Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody
[EP1254] (ab76003)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human gastric adenocarcinoma tissue labeling MMP9 with unpurified ab76003 at a dilution of 1/100.

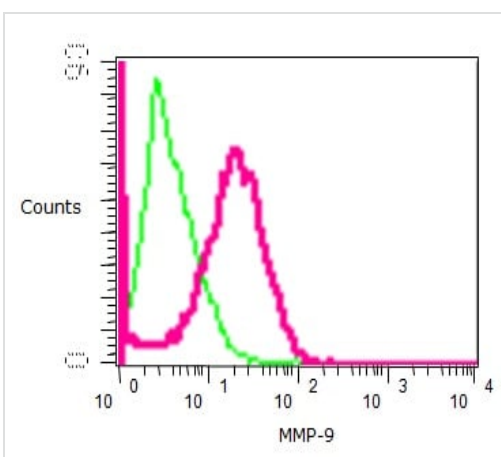
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-MMP9 antibody [EP1254] (ab76003)

Unpurified ab76003 staining MMP9 in U87-MG cells treated with domoic acid (**ab120338**), by ICC/IF. Increase of MMP9 expression correlates with increased concentration of domoic acid, as described in literature.

The cells were incubated at 37°C for 6h in media containing different concentrations of **ab120338** (domoic acid) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with unpurified ab76003 (1/200) dilution was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight® 488 anti-rabbit polyclonal antibody (**ab96899**) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Flow Cytometry (Intracellular) - Anti-MMP9 antibody [EP1254] (ab76003)

Overlay histogram showing permeabilized A431 (Human epidermoid carcinoma cell line) cells stained with unpurified ab76003 (pink line).

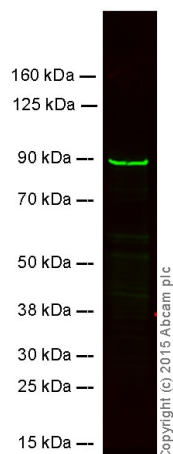
Negative control antibody (green line) was rabbit IgG.

OL-RD Scanning - Anti-MMP9 antibody [EP1254] (ab76003)

Equilibrium disassociation constant (K_D)

[Learn more about \$K_D\$](#)

[Click here to learn more about \$K_D\$](#)



Western blot - Anti-MMP9 antibody [EP1254]
(ab76003)

All lanes : Anti-MMP9 antibody [EP1254] (ab76003) at 1.5 µg/ml

All lanes : Recombinant Human MMP9 protein (Proenzyme)
([ab82955](#))

Lysates/proteins at 0.1 µg per lane.

Secondary

All lanes : Goat anti-rabbit at 1/10000 dilution

Predicted band size: 78 kDa

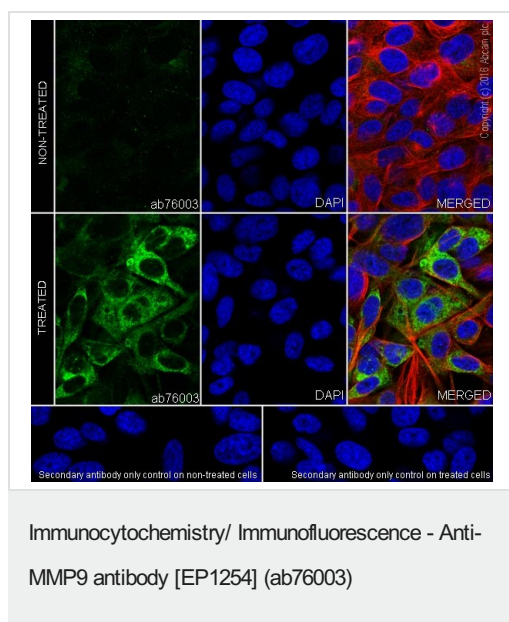
Observed band size: 89 kDa

Running buffer: MOPS.

Conditions: Denatured/reduced.

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes.

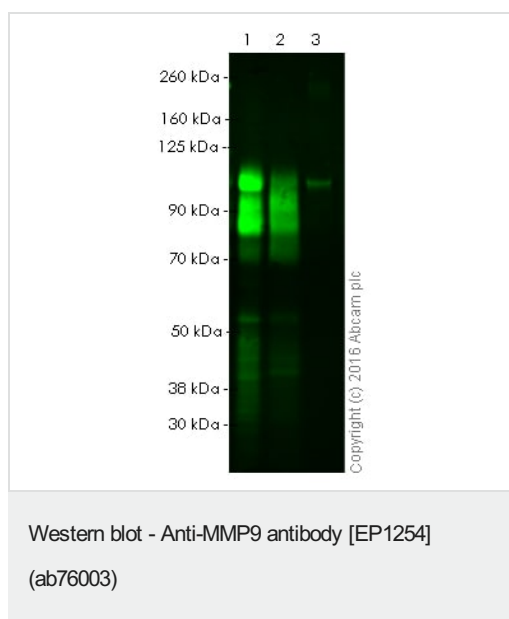
The membrane was then blocked for an hour before being incubated with ab76003 (rabbit-anti MMP9; 1.5 µg/mL) for 48 hours at 4°C. Before imaging, antibody binding was detected using infrared-labeled goat anti-rabbit (green) at 1:10,000 dilutions for 1 hour at room temperature.



Immunocytochemistry/Immunofluorescence analysis of U-2 OS (human osteosarcoma) cells labeling MMP9 with ab76003 at 1/500 (4.3 µg/mL). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000, 2 µg/mL) was used as the secondary antibody. Cells were counterstained with **ab195889** Anti-Alpha Tubulin antibody [DM1A] (1/200, 2.5 µg/mL) - Microtubule Marker (Alexa Fluor® 594). DAPI (blue) was used as a nuclear counterstain.

Confocal image showing cytoplasmic staining on U-2 OS cells, the expression increased after treatment with TPA (200 nM) for 24 hours (middle panel).

Secondary Only Control: PBS was used instead of the primary antibody as the negative control with both TPA treated and untreated U-2 OS cells.



Lanes 1-2 : Anti-MMP9 antibody [EP1254] (ab76003) at 5 µg

Lane 3 : Anti-MMP9 antibody [EP1254] (ab76003) at 5 µg/ml

Lane 1 : Native human MMP9 protein (dimer) (**ab168863**)

Lane 2 : Native human MMP9 protein (Proenzyme, monomer) (**ab157344**)

Lane 3 : Native Mouse MMP9 protein (**ab39309**)

Lysates/proteins at 0.1 µg per lane.

Secondary





All lanes : Infrared labeled goat anti-rabbit (green) antibody at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 78 kDa

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour before being incubated with anti-MMP9 antibody [EP1254] (ab76003; 5 microgram per mL) overnight at 4°C. Antibody binding was detected using infrared labeled goat anti-rabbit (green) antibody (diluted 1:20000) for 1 hour at room temperature before imaging.

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-MMP9 antibody [EP1254] (ab76003)

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