abcam

Product datasheet

Anti-MMP9 antibody [EP1254] ab76003

Recombinant RabMAb

12 Abreviews 451 References 15 Images

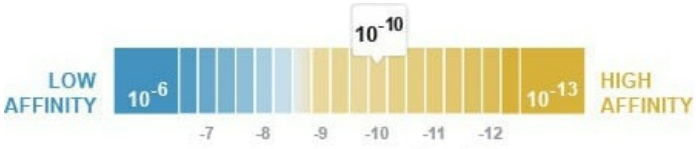
Overview

Product name	Anti-MMP9 antibody [EP1254]
Description	Rabbit monoclonal [EP1254] to MMP9
Host species	Rabbit
Specificity	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.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB, IHC-P
Species reactivity	**Reacts with:** Rat, Human, Recombinant fragment
Immunogen	Synthetic peptide within Human MMP9 aa 100-200. The exact sequence is proprietary. Database link: **P14780**
Positive control	WB: U937, HL60 and TPA treated HT1080 cell lysates, human and rat lung, spleen and lymph node tissue lysate. IHC-P: Human gastic adenocarcinoma and spleen tissue. ICC/IF: U-2 OS and domoic acid-treated U87-MG cells. Flow Cyt (intra): A431 cells.
General notes	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 **here (downloadable copy)**. 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. 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**.

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.
Dissociation constant (K_D)	$K_D = 1.58 \times 10^{-10}$ M



Learn more about K_D

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	EP1254
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab76003 in the following tested applications.

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

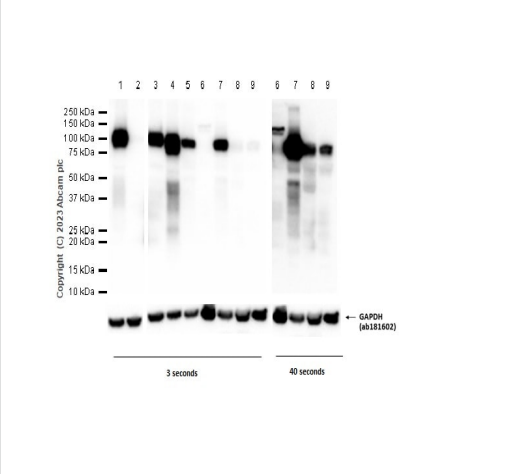
Application	Abreviews	Notes
Flow Cyt (Intra)		1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		1/250 - 1/500.
WB	★★★★☆ (7)	1/1000 - 1/20000. Detects a band of approximately 92 kDa (predicted molecular weight: 78 kDa). Based on our preliminary 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 **IHC antigen retrieval protocols**. **For unpurified use at 1/100 - 1/250.**

Target

Function 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.

Tissue specificity	Produced by normal alveolar macrophages and granulocytes.
Involvement in disease	Intervertebral disc disease Metaphyseal anadysplasia 2
Sequence similarities	Belongs to the peptidase M10A family. Contains 3 fibronectin type-II domains. Contains 4 hemopexin repeats.
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	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.
Cellular localization	Secreted, extracellular space, extracellular matrix.

Images



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

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

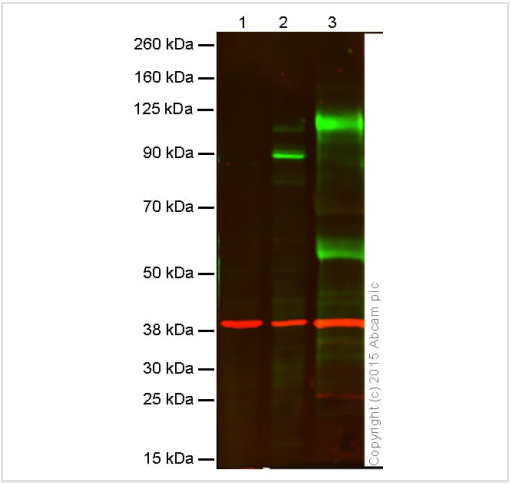
Predicted band size: 78 kDa
Observed band size: 84-92 kDa

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 because MMP9 expression level is low in normal brain and would be increased in abnormal conditions like injury (PMID: 31198417).



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

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

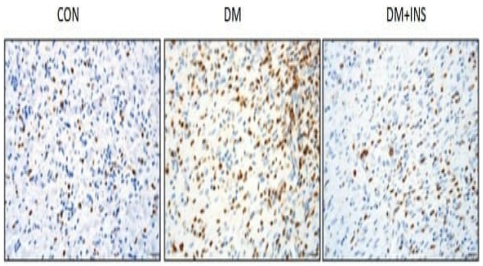
Secondary
All lanes : Goat anti-rabbit at 1/10000 dilution

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.



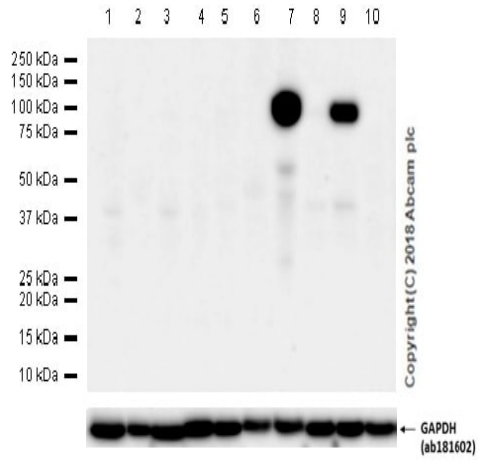
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody [EP1254] (ab76003)

Representitive 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 (8mm^2) 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 N_2 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.



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

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

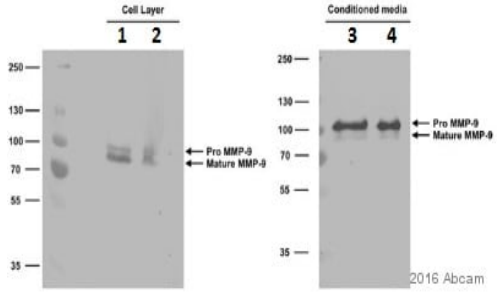
Predicted band size: 78 kDa
Observed band size: 84-82 kDa

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

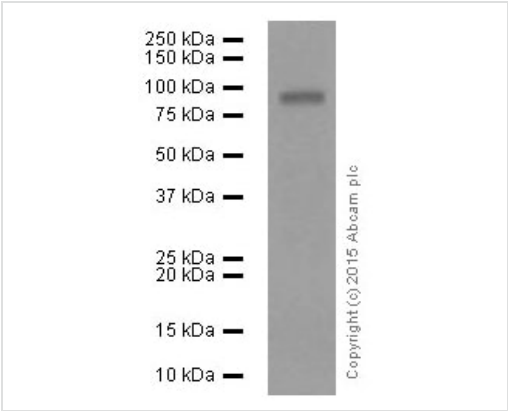
Lanes 1-2 : HTB94 (human chondrosarcoma cell line) cell lysate
Lanes 3-4 : HTB94 (human chondrosarcoma cell line) conditioned media

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

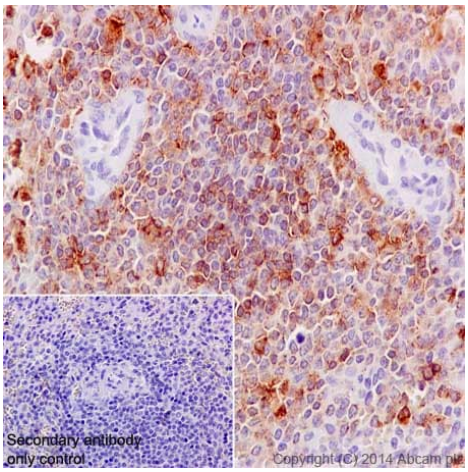
Secondary

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.

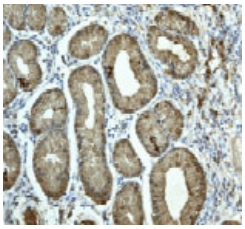


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody [EP1254] (ab76003)

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).

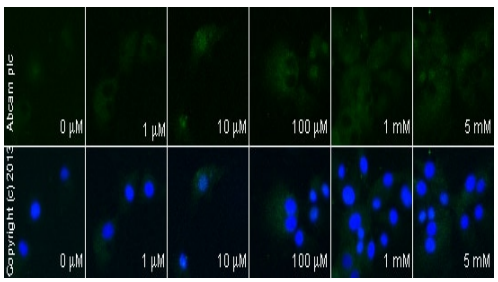
Counterstained with hematoxylin.



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.

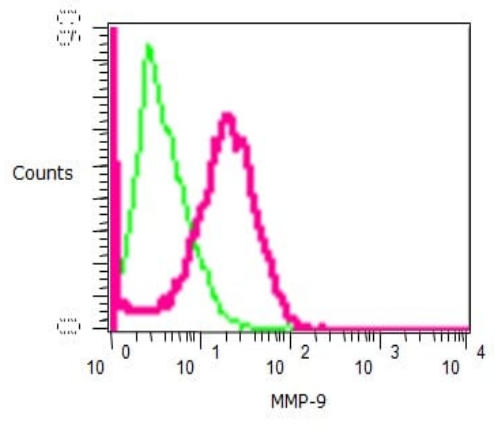
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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).

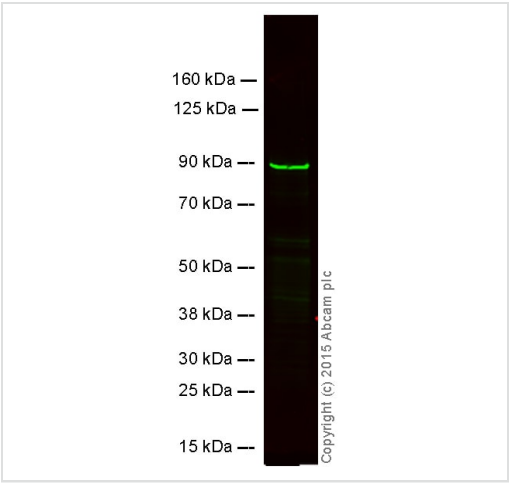
Negative control antibody (green line) was rabbit IgG.

OI-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

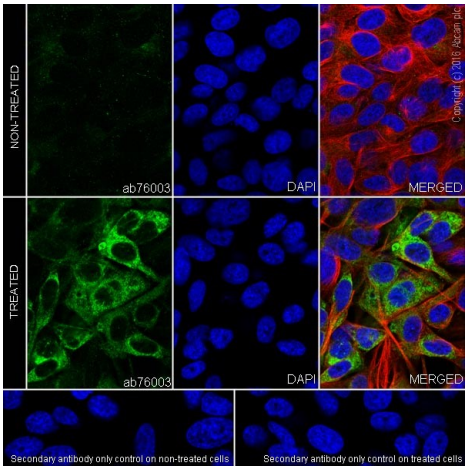
Predicted band size: 78 kDa

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 ug/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.

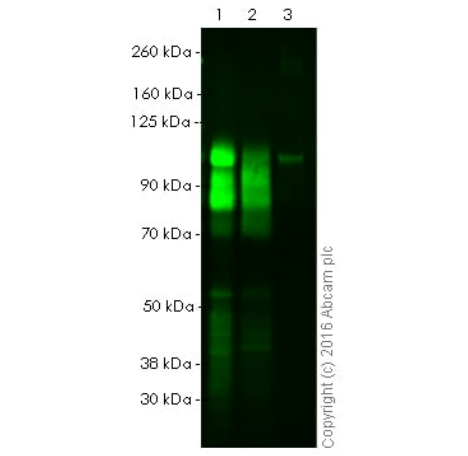


Immunocytochemistry/ Immunofluorescence - Anti-MMP9 antibody [EP1254] (ab76003)

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 onU-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.



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

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**)

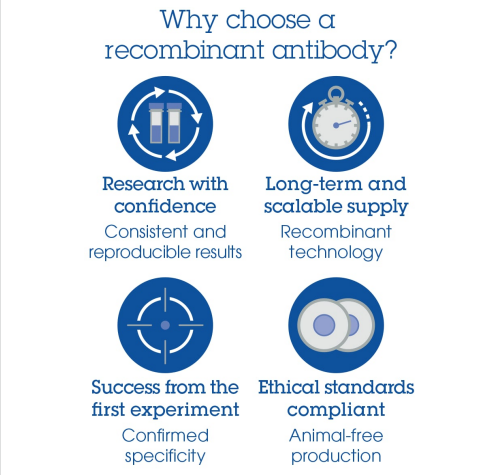
Lysates/proteins at 0.1 µg per lane.

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.



Anti-MMP9 antibody [EP1254] (ab76003)

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