abcam

ab76003

Anti-MMP9 antibody [EP1254]

Anti-MMP9 antibody [EP1254] (ab76003) is a rabbit monoclonal antibody detecting MMP9 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Rat,**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 550 publications
- Trusted since 2009

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP1254
Purification technique	Affinity purification Protein A
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. 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).
Dissociation constant	1.58 x 10^{-10} M
Concentration	2.02 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	**For unpurified use at 1/100 - 1/250.** Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

WB

Tested

Species	Recombinant full length protein - Human
Dilution info	1/1000 - 1/20000
Notes	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.

Species	Rat
Dilution info	1/1000 - 1/20000

Notes	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.

Species	Human
Dilution info	1/1000 - 1/20000
Notes	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.

ICC/IF

Tested

Species	Human
Dilution info	1/250 - 1/500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/1000
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Recombinant full length protein - Human
Dilution info	-
Notes	-

Target data

See full target information MMP9

Function	Matrix metalloproteinase that plays an essential role in local proteolysis of the extracellular matrix and in leukocyte migration (PubMed:12879005, PubMed:1480034, PubMed:2551898). Could play a role in bone osteoclastic resorption (By similarity). Cleaves KiSS1 at a Gly-|-Leu bond (PubMed:12879005). Cleaves NINJ1 to generate the Secreted ninjurin-1 form (PubMed:32883094). Cleaves type IV and type V collagen into large C-terminal three quarter fragments and shorter N-terminal one quarter fragments (PubMed:1480034). Degrades fibronectin but not laminin or Pz-peptide.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Product Specifications
Anti-MMP9 antibody [EP1254] (ab76003) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, WB in human, rat, samples.
Anti-MMP9 antibody [EP1254] (ab76003) specifically detects MMP9 (UniProt ID: P14780; Molecular weight: 1kDa) and is sold in 100 µL and 1 mL selling sizes.

Quality and Validation
Abcam's high quality manufacturing and validation processes ensure Anti-MMP9 antibody [EP1254] (ab76003) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
Anti-MMP9 antibody [EP1254] (ab76003) has been cited over 551 times in peer reviewed journals and is trusted by the scientific community.
Anti-MMP9 antibody [EP1254] (ab76003) has 12 independent reviews from customers.

Related Products
Antibody clone EP1254 is also available pre-conjugated to a variety of labels for your convenience - PE, APC, HRP, Alkaline Phosphatase, Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 594, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 750 (ab305635, ab305636, ab305637, ab308802, ab309755, ab310122, ab310533, ab312063, ab312540, ab321706).

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The MMP-9 protein also known as matrix metalloproteinase-9 or gelatinase B functions as an enzyme involved in the breakdown of extracellular matrix proteins. It consists of a zinc ion at its active site and facilitates the degradation of type IV and V collagens fibronectin and elastin. MMP-9 has a molecular weight of approximately 92 kDa and is expressed in a variety of tissues including the liver lungs and immune cells. This enzyme can exist in monomer and dimer forms with its activity regulated by tissue inhibitors of metalloproteinases (TIMPs).
Biological function summary	Matrix metalloproteinase-9 profoundly influences tissue remodeling inflammation and angiogenesis. It does not form part of a larger protein complex but interacts closely with substrates in the extracellular matrix. MMP-9 plays important roles in processes such as embryogenesis reproduction and wound healing through the remodeling of surrounding tissues. The MMP-9 protein is often measured using

	assays like ELISA or analyzed via techniques such as Western blotting to determine its expression levels and activity in different biological contexts.
Pathways	MMP-9 participates in the matrix metalloproteinase pathway and the NF-kB signaling pathway. These pathways are essential in orchestrating processes such as tissue remodeling and inflammatory responses. MMP-9 acts alongside other matrix metalloproteinases including MMP-2 and is regulated by factors like cytokines growth factors and hormones ensuring the precision of extracellular matrix degradation during physiological processes.
Associated diseases and disorders	Matrix metalloproteinase-9 has associations with cancer invasion and metastasis as well as chronic inflammatory diseases like rheumatoid arthritis. In cancer upregulated MMP-9 expression aids tumor cells in breaching the basement membrane facilitating metastasis. The disorder-associated pathways often involve interactions with proteins like vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-beta). Understanding MMP-9's role in these conditions can contribute to developing targeted therapies that inhibit its activity potentially mitigating disease progression.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

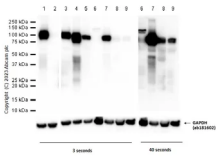
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

13 product images



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

MMP9 Western blot staining using rabbit Anti-MMP9 antibody

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).

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

Lane 1:
Human lung tissue lysate at 20 µg

Lane 2:
Human brain tissue lysate at 20 µg

Lane 3:
Human spleen tissue lysate at 20 µg

Lane 4:
Human lymph node tissue lysate at 20 µg

Lane 5:
Rat lung tissue lysate at 20 µg

Lane 6:
Rat brain tissue lysate at 20 µg

Lane 7:
Rat spleen tissue lysate at 20 µg

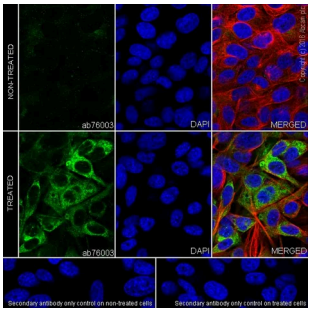
Lane 8:
Rat kidney tissue lysate at 20 µg

Lane 9:
Rat lymph node tissue lysate at 20 µg

Secondary

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

Predicted band size: 29 kDa



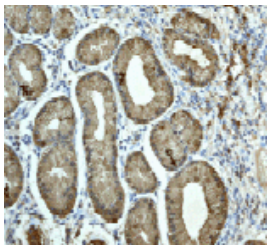
Immunocytochemistry/ Immunofluorescence - Anti-MMP9 antibody [EP1254] (ab76003)

MMP9 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-MMP9 antibody

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).

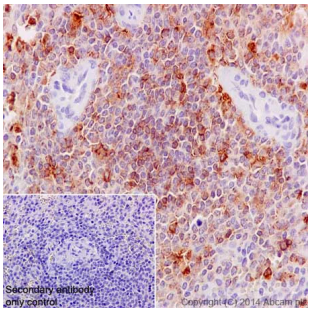
Secondary Only Control: PBS was used instead of the primary antibody as the negative control with both TPA treated and untreated U-2 OS cells.



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

MMP9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human gastric adenocarcinoma tissue using rabbit Anti-MMP9 antibody

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.

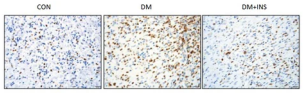


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody [EP1254] (ab76003)

MMP9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MMP9 antibody

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.



Abdollahi et al PLoS One. 2017 Feb 9;12(2):e0170951. doi: 10.1371/journal.pone.0170951. eCollection 2017. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

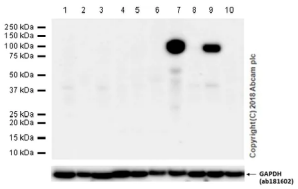
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody [EP1254] (ab76003)

MMP9 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-MMP9 antibody

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 (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.



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

MMP9 Western blot staining using rabbit Anti-MMP9 antibody

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.

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

Lane 1:
LoVo (Human colorectal adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
Huh7 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 4:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
Caco-2 (Human colorectal adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 6:
A549 (Human lung carcinoma epithelial cell) serum starved overnight whole cell lysate at 20 µg

Lane 7:
A549 (Human lung carcinoma epithelial cell) serum starved overnight, then treated with 80nM TPA for 24 hours whole cell lysate at 20 µg

Lane 8:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) serum starved overnight whole cell lysate at 20 µg

Lane 9:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) serum starved overnight, then treated with 200nM TPA for 24 hours whole cell lysate at 20 µg

Lane 10:
HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

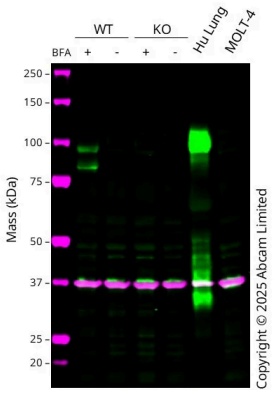
Secondary

All lanes:
Western blot - 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: 60s



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

MMP9 Western blot staining using rabbit Anti-MMP9 antibody

Western blot: Rabbit Monoclonal [EP1254] to MMP9 76003 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH (ab8245) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 90 kDa in Wild-type A549 TPA-treated (80nM, 24h) cell lysates with no signal observed at this size in MMP9 knockout A549 TPA-treated (80nM, 24h) cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-MMP9 antibody [EP1254] (ab76003) at 1/1000 dilution

Lane 1:
Wild-type A549 TPA-treated (80nM, 24h) at 20 µg

Lane 2:
Wild-type A549 Vehicle Control TPA (0nM, 24h) at 20 µg

Lanes 3 - 4:
Western blot - Human MMP9 knockout A549 cell line (ab286527) at 20 µg

Lane 5:
Human Lung at 20 µg

Lane 6:
MOLT-4 at 20 µg

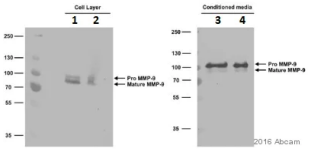
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 78 kDa

Observed band size: 90 kDa



Data courtesy of an anonymous customer review

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

MMP9 Western blot staining using rabbit Anti-MMP9 antibody

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

Lanes 1 - 2:
HTB94 (human chondrosarcoma cell line) cell lysate at 10 µg

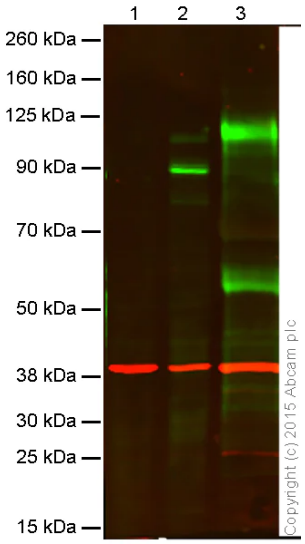
Lanes 3 - 4:
HTB94 (human chondrosarcoma cell line) conditioned media at 10 µg

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)

MMP9 Western blot staining using rabbit Anti-MMP9 antibody

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.

All lanes:
Western blot - 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

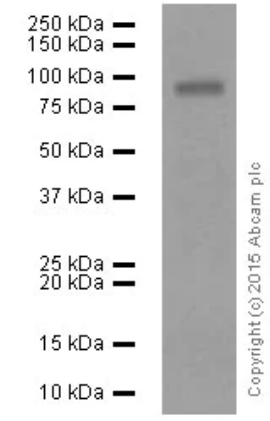
Lane 3:
Human tonsils at 20 µg

Secondary

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

Predicted band size: 78 kDa

Observed band size: 89 kDa



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

MMP9 Western blot staining of Rat kidney tissue lysate using rabbit Anti-MMP9 antibody

Blocking and dilution buffer: 5% NFDM/TBST.
All lanes:
Western blot - Anti-MMP9 antibody [EP1254] (ab76003) at 1/1000 dilution

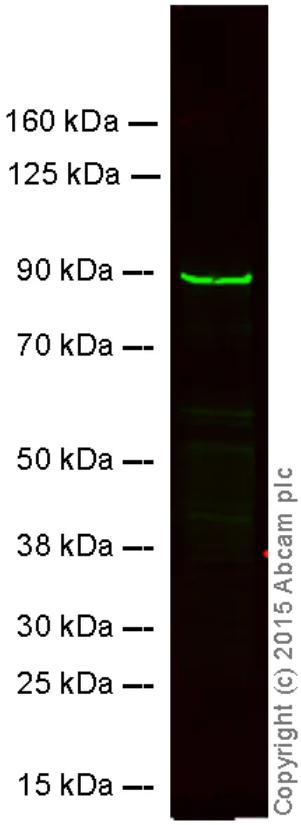
All lanes:
Rat kidney tissue lysate at 10 µg

Secondary

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

Predicted band size: 78 kDa

Observed band size: 84-92 kDa



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

MMP9 Western blot staining using rabbit Anti-MMP9 antibody

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.

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

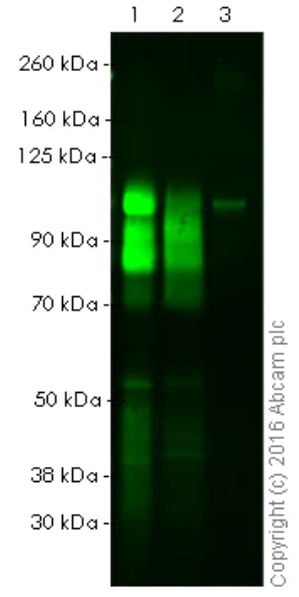
All lanes:
Western blot - Recombinant Human MMP9 protein (Proenzyme) (ab82955) at 0.1 µg

Secondary

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

Predicted band size: 78 kDa

Observed band size: 89 kDa



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

MMP9 Western blot staining using rabbit Anti-MMP9 antibody

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

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

Lane 1:
Western blot - Native human MMP9 protein (dimer) (ab168863) at 0.1 µg

Lane 2:
Western blot - Native human MMP9 protein (Proenzyme, monomer) (ab157344) at 0.1 µg

Lane 3:
Western blot - Recombinant Mouse MMP9 protein (ab39309) at 0.1 µg

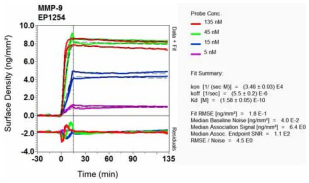
Secondary

All lanes:

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

Performed under reducing conditions.

Predicted band size: 78 kDa



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

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody.
Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.