**Product datasheet**

**Anti-MMP9 antibody ab38898**

*44 Abreviews 240 References 8 Images*

**Overview**

**Product name**  
Anti-MMP9 antibody

**Description**  
Rabbit polyclonal to MMP9

**Host species**  
Rabbit

**Specificity**  
The antibody binds to Gelatinase-B, but does not cross react with the other MMP family members (MMP-1, MMP-2, MMP-3). In our hands, we observe a weaker signal in WB in human samples compared to mouse samples (BLAST of full length mouse protein sequence showed 72% homology with the Human MMP9 sequence).

**Tested applications**  
Suitable for: IHC-P, IHC-Fr, WB, IP, ELISA, ICC/IF, ICC, IHC-FoFr

**Species reactivity**  
Reacts with: Mouse, Rat, Dog, Human

**Immunogen**  
Full length protein corresponding to Mouse MMP9.

**Positive control**  
HL60 cell lysate. U937 cell lysate. HT1080 cell lysate. Raw 264.7 cell lysate (LPS treated)

**Properties**

**Form**  
Liquid

**Storage instructions**  
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Store at -20°C.

**Storage buffer**  
Preservative: 0.05% Sodium azide  
Constituent: 50% Glycerol

**Purity**  
Immunogen affinity purified

**Clonality**  
Polyclonal

**Isotype**  
IgG

**Applications**

Our Abpromise guarantee covers the use of ab38898 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-P</td>
<td>★★★☆☆☆☆☆</td>
<td>1/100 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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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.

Application | Abreviews | Notes
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IHC-Fr | ⭐⭐⭐⭐ ⭐ | 1/1000.
(see Abreview submitted by Greg Gibson)
We recommend using Goat Anti-Rabbit IgG H&L (Cy3 ®) preadsorbed (ab6939) secondary antibody

WB | ⭐⭐⭐⭐ ⭐ | 1/1000. Detects a band of approximately 92 kDa. When using colorimetric substrates such as BCIP/NBT use at a 1/5000 dilution (for chemiluminescent substrates). Detects a band of approximately 92-95 kDa (pro-form) and 82kDa (active form) (Human samples). Mouse MMP9 is larger, and on SDS PAGE gels runs about 102-105 kDa. Dilution optimised using Chromogenic detection.

IP |  | Use at an assay dependent concentration.

ELISA | ⭐⭐⭐⭐ ⭐ | Use at an assay dependent concentration.

ICC/IF | ⭐⭐⭐⭐ ⭐ | 1/500.

ICC |  | Use at an assay dependent concentration.

IHC-FoFr | ⭐⭐⭐⭐ ⭐ | Use at an assay dependent concentration. PubMed: 19295156

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.

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Images
All lanes: Anti-MMP9 antibody (ab38898) at 2 µg/ml

Lane 1: Natural human MMP9 protein (Proenzyme, monomer) (ab157344)
Lane 2: Recombinant Mouse MMP9 protein (ab39309)

Lysates/proteins at 0.1 µg per lane.

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

Performed under reducing conditions.

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 (ab38898; 2 microgram per mL) overnight at 4°C. Antibody binding was detected using infrared labelled goat anti-rabbit (green) antibody (diluted 1:20000) for 1 hour at room temperature before imaging.
Anti-MMP9 antibody (ab38898) + Human MMP9

**Observed band size:** 88.92 kDa

Ab38898 detects a band at 92 Kd (pro-form) and a band at 88 Kd (active form). Mouse MMP9 is slightly larger than human MMP9, and the antibody detects a band at about 105 Kd. It is recommended to concentrate samples by Gelatin-agarose affinity chromatography prior to Western blot usage. A recommended starting concentration for Western blots is 1:1000 when using colorimetric substrates such as BCIP/NBT, and 1:5000 for chemiluminescent substrates. Higher concentration of antibody may be needed for non-human samples.
All lanes: Anti-MMP9 antibody (ab38898) at 5 µg/ml

Lane 1: Recombinant Human MMP9, His tagged (ab82955) at 0.1 µg
Lane 2: U937 whole cell lysate at 100 µg
Lane 3: U937 whole cell lysate - treated with PMA and Brefeldin (24 hour treatment) at 100 µg
Lane 4: Raw 264.7 (Mouse) whole cell lysate at 100 µg
Lane 5: Raw 264.7 (Mouse) whole cell lysate - treated with LPS (6 hour treatment, 1ug/mL) at 100 µg

Performed under reducing conditions.

ab38898 detects recombinant Human MMP9 running at ~85 kDa, and endogenous full-length MMP9 in LPS-stimulated cells at ~100 kDa. This antibody also detects a band at 90 kDa in U937 PMA-treated cells.

ab38898 was incubated at 5 ug/mL and ab8245 (loading control to GAPDH) was diluted at 0.1 ug/mL and both were incubated for 48 hours at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.
**Western blot - Anti-MMP9 antibody (ab38898)**

This image is courtesy of an anonymous Abreview.

**All lanes**: Anti-MMP9 antibody (ab38898) at 1/1000 dilution

**Lanes 1-2**: Human lung tissue lysate at 100 µg with 10% Milk for 1 hour at room temperature

**Lanes 3-4**: MMP-9 KO mice tissue lysates with 10% Milk for 1 hour at room temperature

**Secondary**

**All lanes**: HRP-conjugated donkey anti-rabbit polyclonal at 1/1000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Specific observed bands 95-100 kDa

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**Immunocytochemistry/ Immunofluorescence - Anti-MMP9 antibody (ab38898)**

ab38898 staining MMP9 (red) in Mouse Neutrophils and Monocytes cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 2%BSA + 0.2% tritonX100 in PBS. Samples were incubated with primary antibody (1/200 in 2%BSA + 0.2% tritonX100 in PBS) for 25 minutes at 23°C. An Alexa Fluor® 568-conjugated Donkey anti-rabbit IgG polyclonal (1/1000) was used as the secondary antibody. DAPI is stained blue.

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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody (ab38898)

This image is courtesy of an anonymous Abreview.

ab38898 staining MMP9 in Mouse Pancreatic carcinoma tissue sections by IHC-P (formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 1 hour at room temperature. Antigen retrieval was by heat mediation in citric acid (pH6). Samples were incubated with primary antibody (1/100) in 1% Aurion BSA for 12 hours. An HRP-conjugated Donkey polyclonal to rabbit IgG (1/100) was used as secondary antibody.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-MMP9 antibody (ab38898)


ab38898 staining MMP9 in 6 month-old transgenic zebrafish pancreas (lhha overexpression) by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Sections were incubated with primary antibody (1/500) and HRP-conjugated secondary antibody colored using DAB solution. Slides were counterstained with hematoxylin.
Immunohistochemistry (Frozen sections) - Anti-MMP9 antibody (ab38898)

ab38898 at a 1/1000 dilution staining mouse heart tissue by Immunohistochemistry (Frozen sections). The tissue was removed from a mouse, rinsed in PBS and slowly frozen in supercooled isopentane. 14um sections were made using a cryostat. The sections were acetone fixed and blocked in 2% BSA prior to incubation with the MMP9 antibody. Goat Anti-Rabbit IgG H&L (Cy3 ®) preadsorbed (ab6939) was used as the secondary antibody. In the image: red staining = MMP9, blue staining = nuclei, green = gelatinase activity.

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