ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

Matrix metalloproteinase-9 antibody

□ Cat # MMP9-A	Rabbit anti-Mouse Matrix metalloproteinase-9 (MMP-9) antibody	**SIZE**: 100 µg
□ Cat # MMP9-C	Recombinant MMP-9 protein control for Western blotting	**SIZE**: 200 µl

The matrix metalloproteinases (MMPs) are a family of proteases that target many extracellular proteins including other proteases, growth factors, cell surface receptors, and adhesion molecules. Among the family members, MMP-2, MMP-3, MMP-7, and MMP-9 have been characterized as important factors for normal tissue remodeling during embryonic development, wound healing, tumor invasion, angiogenesis, carcinogenesis, and apoptosis. MMP9 (matrix metallopeptidase 9, GELB, CLG4B) is secreted as a 92kDa zymogen and cleavage of pro-MMP9 results in the active enzyme with a molecular weight of 82kDa. MMP9 has a gelatin-binding domain consisting of three fibronectin type II units, a catalytic domain containing the zinc-binding site, a proline-rich type V collagen-homologous domain and a hemopexin-like domain. MMP9 is produced by monocytes, macrophages, neutrophils, keratinocytes, fibroblasts, osteoclasts and endothelial cells, and is involved in inflammatory responses, tissue remodeling, wound healing, tumor growth and metastasis. MMP9 is supplied by bone marrow-derived cells and contributes to skin carcinogenesis. Further, MMP9 degrades type IV and V collagens. Elevated MMP9 is associated with progression of idiopathic atrial fibrillation and aortic aneurysm.

Source of Antigen or Antibodies

Uniprot: P41245

Host: Rabbit

Clonality: Polyclonal

Purification: Ammonium sulfate followed by peptide affinity purification

Immunogen: 16 amino acid synthetic peptide within region 150-250 from Mouse MMP-9

Species Reactivity: Mouse and Rat

Cross reactivity: The extracellular peptide used as an immunogen exhibits 88% homology with Rat. 81% with Human, Goat, Cat, Dog, Pig, and Monkey. Reactivity has only been confirmed in Mouse and Rat samples.

Subcellular Location: Extracellular or secreted.

Alternative names: 92 kDa gelatinase, 92 kDa type IV collagenase, Gelatinase B

Recommended Secondary Antibody: Goat anti-Rabbit IgG-HRP (**ADI cat#20320)**

Negative Control: Non-immune Rabbit IgG (**ADI cat# 20009-1**).

Form & Storage of Antibodies

□**Affinity pure IgG Solution**
Concentration: 0.5 mg/ml Volume: 200 µl
Supplied in PBS, pH 7.4 + 0.1% BSA
The antibody can be made available carrier free or conjugated to HRP, Biotin, or FITC on request

□ **Lyophilized powder**
Reconstitute powder in 200 µl distilled water to 0.5 mg/ml

Storage:
Short-term: 4°C for 1 month

Long-term: at –20°C or below in suitable aliquots after reconstitution for 1 year. Do not expose to multiple free/thaw cycles or store working, diluted solutions.

Recommended Usage

ELISA: Assay dependent concentration. Typically, between 0.1-2.0 µg/ml for capture/detection antibody.

Western Blotting: 0.5-2.0 µg/ml

MMP9-C: Contains a recombinant HEK293 expressed full length Mouse MMP-9 protein at a concentration of 1 ng/µl in Laemmli buffer (62.5 mM Tris-HCL, pH 6.8, 2% SDS, 10% glycerol, 5% BME, and 0.002% bromphenol blue). Heat for 5 minutes at 95°C then load 1-10 µl. Store at -20°C in suitable size aliquots, do not expose to multiple free/thaw cycles. **Note:** Due to the addition of tags, the protein appears slightly larger than native MMP-9.

IHC-P: 1-10 µg/ml. QC tested using 10 mM sodium citrate, pH 6 antigen retrieval. The antibody may work better with other retrieval solutions or no retrieval.

The above concentrations are a *suggestion*, user's must optimize their assay based on their own conditions. The antibody may work in other applications such as Immunocytochemistry or IP. These methods have not been tested by ADI.

This product is for In vitro research use only.

Related materials available from ADI

Catalog#	Description
VIM21-A	Rabbit Anti-Mouse Vimentin (Ser299) antibody
VIM31-A	Rabbit Anti-Mouse Vimentin (Ser214) antibody
BCL11-A	Rabbit Anti-Human BCL-2 antibody
BCL21-A	Rabbit Anti-Mouse BCL-2 antibody
GFAP11-A	Rabbit anti-Mouse phospho Glial fibrillary acidic protein (S266) antibody
GFAP21-A	Rabbit anti-Mouse Glial fibrillary acidic protein (GFAP) antibody
MMP-9	191701IA

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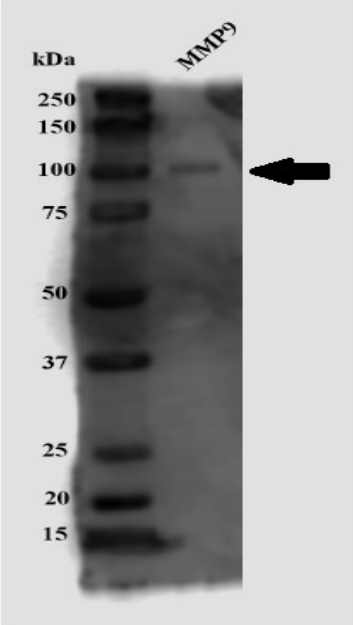

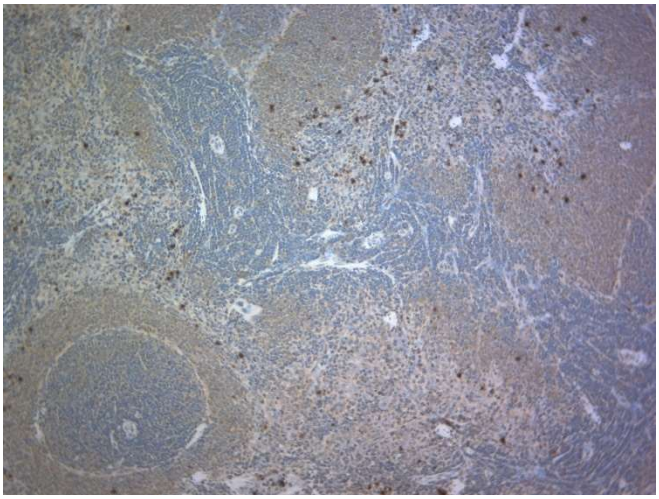

Western blotting: 5 ng of recombinant HEK293 expressed Mouse MMP-9 (**MMP9-C)** was heated for 5 minutes at 95ºC then electrophoretically separated on a 10% SDS-PAGE gel. The gel was run at 100V for ~1 hour and 30 minutes then transferred to a 0.2 µm nitrocellulose membrane using the 'Mixed MW' settings on a Transblot Turbo (Biorad). The blot was blocked for 1 hour at room temperature with Fish plasma (Aquablock, EastCoastBio). **MMP9-A** was diluted with TBST+0.1% BSA to 1 ug/ml and incubated overnight at 4ºC. The blot was washed with TBS-T 3 times for 5 minutes each. Goat anti-rabbit IgG HRP (**ADI cat#20320**) was diluted in TBST+0.1% BSA at a 1:10,000 dilution (50 ng/ml) then incubated for 1 hour at room temperature. The blot was washed 3 times with TBS-T for 5 minutes each. The blot was then incubated with ADI Femto ECL substrate (**ADI cat#80210**) for 5 minutes and imaged on a CCD imaging system (C-DiGit, LI-COR).

Predicted band size: 80.5 kDa
Observed band size: ~105 kDa. Due to variable glycosylation the protein may migrate higher than then the predicted MW.

Immunohistochemistry: Rat spleen slide was heated for 20 minutes at 60ºC then deparrafinized. Antigen retrieval was performed for 10 minutes at 95ºC in a microwave using 10 mM pH 6, sodium citrate buffer. The slide was then cooled for 20 minutes at room temperature before being blocked for 30 minutes with 2.5% normal goat serum. **MMP9-A** was diluted to 5 µg/ml in TBST+0.1% BSA and incubated overnight at 4ºC. The slides were then washed twice and incubated with 3% hydrogen peroxide for 10 minutes to quench endogenous peroxidase. The slide was washed then incubated with Goat anti-Rabbit IgG HRP polymer detection reagent for 30 minutes at room temperature. The slide was washed twice, incubated with DAB for 3 minutes, washed with distilled water, then counterstained for 1 minute with Gil's II Hematoxylin before being cover-slipped.