## CATALOG NO: EK0465

## LOT NO: LOT#\_\_\_\_\_\_\_

## INTENDED USE

For the quantitation of Human MMP9 concentrations in cell culture supernatants, serum, plasma (heparin) , saliva and urine.

## BACKGROUND

The 92-kD type IV collagenase is also known as 92-kD gelatinase, type V collagenase, gelatinase B, or matrix metalloproteinase-9 (MMP9). The 72- and 92-kDa type IV collagenases are members of a group of secreted zinc metalloproteases. The matrix metalloproteinases (MMPs) are able to degrade the extracellular matrix and allow angiogenesis and tumor invasion. Gelatinase B, a matrix metalloproteinase that has proteolytic activity against connective tissue proteins, has been suggested to be important in the connective tissue remodeling processes associated with atherogenesis and plaque rupture. MMP-9 is predominantly expressed in neutrophils, macrophages, and mast cells, rather than in oncogene-positive neoplastic cells. The polymorphism of MMP-9 acts as a genetic factor for the development of smoking-induced pulmonary emphysema. The standard product used in this kit is recombinant human MMP-9 with the molecular mass of 95KDa. The detected MMP-9 includes zymogen and active enzyme. 75 Publications Citing This Product PubMed ID: 10.3892/mmr.2019.10330, Calcium‑sensing receptor promotes high glucose‑induced myocardial fibrosis via upregulation of the TGF‑beta1/Smads pathway in cardiac fibroblasts PubMed ID: 10.1161/STROKEAHA.115.011230, Combining Growth Factor and Bone Marrow Cell Therapy Induces Bleeding and Alters Immune Response After Stroke in Mice PubMed ID: 10.1186/s12931-020-01426-9, Shp2 positively regulates cigarette smoke-induced epithelial mesenchymal transition by mediating MMP-9 production

## PRINCIPLE OF THE ASSAY

The Innovative Research Human MMP9 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human MMP9 with a 96-well strip plate that is pre-coated with antibody specific for MMP9. The detection antibody is a biotinylated antibody specific for MMP9. The capture antibody is polyclonal antibody from goat and the detection antibody is polyclonal antibody from goat. The kit includes Human MMP9 protein as standards. To measure Human MMP9, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human MMP9 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human MMP9 in the sample.

## OVERVIEW

\*The sensitivity or the minimum detectable dose (MDD) is the lower limit of the target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

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| Product Name | Human MMP-9 ELISA Kit |
| Reactive Species | This kit is for the detection of Human MMP9. No significant cross-reactivity or interference between MMP9 and its analogs was observed. This claim is limited by existing techniques; therefore, cross- reactivity may exist with untested analogs. |
| Size | 96 wells/kit, with removable strips. |
| Description | Human MMP-9 ELISA Kit (96 Tests). Quantitate Human MMP9 in cell culture supernatants, serum, plasma (heparin) , saliva and urine. Sensitivity: 5pg/ml. The brand indicates this is a premium quality ELISA kit. Each kit delivers precise quantification, high sensitivity, and excellent reproducibility. Only our most reliable and effective kits qualify as , guaranteeing top-tier results for your assays. |
| Sensitivity | 5 pg/ml |
| Detection Range | 156 pg/ml - 10,000 pg/ml |
| Storage Instructions | Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature. Freeze upon receiving.) |
| Uniprot ID | P14780 |

## TECHNICAL DETAILS

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| --- | --- |
| Capture/Detection Antibodies | The capture antibody is polyclonal antibody from goat and the detection antibody is polyclonal antibody from goat. |
| Specificity | Natural and recombinant Human MMP9 |
| Standard Protein | Expression system for standard: NS0; Immunogen sequence: A20-D707 |
| Cross-reactivity | This kit is for the detection of Human MMP9. No significant cross-reactivity or interference between MMP9 and its analogs was observed. This claim is limited by existing techniques; therefore, cross- reactivity may exist with untested analogs. |

## PREPARATIONS BEFORE ASSAY

Please read the following instructions before starting the experiment. Read this manual in its entirety in order to minimize the chance of error. Confirm that you have the appropriate non-supplied equipment available. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation). To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended. Before using the kit, spin tubes to bring down all components to the bottom of the tubes. Don’t let the 96-well plate dry out since this will inactivate active components on the plate. Don’t reuse tips and tubes to avoid cross-contamination. Avoid using the reagents from different batches together. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

## KIT COMPONENTS/MATERIALS PROVIDED

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| **Component** | **Quantity** |

Anti-Human MMP9 Pre-coated 96-well Strip Microplate1Human MMP9 Standard2Human MMP9 Biotinylated Antibody (100x)1Avidin-Biotin-Peroxidase Complex (100x)1Sample Diluent1Antibody Diluent1Avidin-Biotin-Peroxidase Diluent1Color Developing Reagent (TMB)1Stop Solution1Wash Buffer (25x)1Plate Sealers4

## MATERIALS REQUIRED BUT NOT PROVIDED

• Microplate reader capable of reading absorbance at 450 nm. Incubator. • Automated plate washer (optional) • Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for a large numbers of samples.

## REAGENT PREPARATION

Bring all reagents to room temperature before use. Wash Buffer: Dilute Wash Buffer (25X) with distilled water. For example, if preparing 500 ml of Wash Buffer, dilute 20 ml of Wash Buffer (25X) into 480 ml of distilled water. Standard: Reconstitute the standard with standard diluent according to the label instructions. This reconstitution produces a stock solution. Let the standard stand for a minimum of 15 minutes with gentle agitation prior to making dilutions. Detection Reagent A and B: Dilute to the working concentration using Assay Diluent A and B, respectively.

## SAMPLE PREPARATION

## DILUTION OF STANDARD

1. Label 7 tubes, one for each standard: 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, and 62.5 pg/ml. 2. Pipette 300 µl of the Sample Diluent into each tube. 3. Pipette 300 µl of the reconstituted standard into the first tube and mix to create the 4000 pg/ml standard. 4. Pipette 300 µl from the 4000 pg/ml tube into the second tube and mix to create the 2000 pg/ml standard. 5. Continue this process for the remaining tubes. 6. The Sample Diluent serves as the zero standard (0 pg/ml).

## TYPICAL DATA / STANDARD CURVE

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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| --- | --- |
| **Concentration (pg/ml)** | **O.D.** |

[{'concentration': '0.0', 'od\_value': '0.117'}]

## INTRA/INTER-ASSAY VARIABILITY

- Intra-Assay Precision: Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision: Three samples of known concentration were tested in separate assays to assess inter-assay precision.

## ASSAY PROTOCOL

1. It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Preparation Before The Experiment, if you have missed this information). Prepare all reagents and working standards as directed previously. Remove excess microplate strips from the plate frame and seal and store them in the original packaging. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the Sample Diluent into the zero well. At least two replicates of each standard, sample, or control is recommended. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min. at 37 °C). Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 100 µl of the prepared 1x Biotinylated Anti-Human MMP9 antibody to each well. Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C). Wash the plate 3 times with the 1x wash buffer: Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash). Repeat steps a-b 2 additional times. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C). Wash the plate 5 times with the 1x wash buffer: Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash). Repeat steps a-b 4 additional times. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.) Add 100 µl of Stop Solution to each well. The color should immediately change to yellow. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm. Assay Protocol Notes Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.

## DATA ANALYSIS

To analyze using manual methods, follow the process below: Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading. It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative O.D. against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data. For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor. Background on MMP9 The 92-kD type IV collagenase is also known as 92-kD gelatinase, type V collagenase, gelatinase B, or matrix metalloproteinase-9 (MMP9). The 72- and 92-kDa type IV collagenases are members of a group of secreted zinc metalloproteases. The matrix metalloproteinases (MMPs) are able to degrade the extracellular matrix and allow angiogenesis and tumor invasion. Gelatinase B, a matrix metalloproteinase that has proteolytic activity against connective tissue proteins, has been suggested to be important in the connective tissue remodeling processes associated with atherogenesis and plaque rupture. MMP-9 is predominantly expressed in neutrophils, macrophages, and mast cells, rather than in oncogene-positive neoplastic cells. The polymorphism of MMP-9 acts as a genetic factor for the development of smoking-induced pulmonary emphysema. The standard product used in this kit is recombinant human MMP-9 with the molecular mass of 95KDa. The detected MMP-9 includes zymogen and active enzyme. 75 Publications Citing This Product PubMed ID: 10.3892/mmr.2019.10330, Calcium‑sensing receptor promotes high glucose‑induced myocardial fibrosis via upregulation of the TGF‑beta1/Smads pathway in cardiac fibroblasts PubMed ID: 10.1161/STROKEAHA.115.011230, Combining Growth Factor and Bone Marrow Cell Therapy Induces Bleeding and Alters Immune Response After Stroke in Mice PubMed ID: 10.1186/s12931-020-01426-9, Shp2 positively regulates cigarette smoke-induced epithelial mesenchymal transition by mediating MMP-9 production html to see all 75 publications. Human MMP-9 ELISA Kit

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