Datasheet for ABIN625060

MMP 9 ELISA Kit

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Overview

Quantity:	96 tests
Target:	MMP 9 (MMP9)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	10-6000 pg/mL
Minimum Detection Limit:	10 pg/mL
Application:	ELISA

Product Details

Purpose:	Human MMP-9 ELISA Kit for cell culture supernatants, heparin treated plasma, and serum samples. EDTA and Citrate are not recommended.
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This ELISA kit shows no cross-reactivity with any of the cytokines tested: Human Angiogenin, BDNF, BLC, ENA-78, FGF-4, IL-1 alpha, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, I-309, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin (OB), MCP-1, MCP-3, MDC, MIP-1 alpha, MIP-1 beta, MIP-1 delta, MMP-1, - 2, -3, -10, PARC, RANTES, SCF, TARC, TGF-beta, TIMP-1, TIMP-2, TNF-alpha, TNF-beta, TPO, VEGF.
Sensitivity:	< 10 pg/mL

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Product Details

Characteristics:	• Strip plates and additional reagents allow for use in multiple experiments • Quantitative protein detection • Establishes normal range • The best products for confirmation of antibody array data
Components:	• Pre-Coated 96-well Strip Microplate • Wash Buffer • Stop Solution • Assay Diluent(s) • Lyophilized Standard • Biotinylated Detection Antibody • Streptavidin-Conjugated HRP • TMB One-Step Substrate
Material not included:	• Distilled or deionized water • Precision pipettes to deliver 2 µL to 1 µL volumes • Adjustable 1-25 µL pipettes for reagent preparation • 100 µL and 1 liter graduated cylinders • Tubes to prepare standard and sample dilutions • Absorbent paper • Microplate reader capable of measuring absorbance at 450nm • Log-log graph paper or computer and software for ELISA data analysis

Target Details

Target:	MMP 9 (MMP9)
Alternative Name:	MMP-9 (MMP9 Products)
Background:	Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade extracellular matrix proteins. MMPs have been linked with a wide array of biological activities and play important roles during organ development and pathological processes. Collectively MMPs are key enzymes for the metabolism of extracellular matrix proteins, including fibrillar and non-fibrillar collagens, fibronectin, laminin and basement membrane or interstitial stroma glycoproteins. Under physiological conditions MMPs are involved in extracellular degradation and breakdown of matrix proteins during normal tissue remodelling processes such as wound healing, pregnancy, and angiogenesis. Human MMP-9 is a 92 kDa glycoprotein that plays a significant role in matrix remodeling, enzyme modulation, and cytokine/growth factor activation. MMP-9 is also known as gelatinase B based on its ability to degrade gelatin. The Human MMP-9 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human MMP-9 pro and active

Target Details

	forms in serum, plasma (Collect plasma using heparin as an anticoagulant. EDTA and Citrate are not recommended), cell culture supernatants and urine. This assay employs an antibody specific for human MMP-9 coated on a 96-well plate. Standards and samples are pipetted into the wells and MMP-9 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human MMP-9 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MMP-9 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%.
Gene ID:	4318
UniProt:	P14780
Pathways:	Cellular Response to Molecule of Bacterial Origin, Positive Regulation of Immune Effector Process, CXCR4-mediated Signaling Events

Application Details

Application Notes:	Recommended Dilution for serum and plasma samples300 - 3,000 fold
Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Prepare all reagents, samples and standards as instructed in the manual. 2. Add 100 µL of standard or sample to each well. 3. Incubate 2.5 h at RT or O/N at 4 °C. 4. Add 100 µL of prepared biotin antibody to each well. 5. Incubate 1 h at RT. 6. Add 100 µL of prepared Streptavidin solution to each well. 7. Incubate 45 min at RT. 8. Add 100 µL of TMB One-Step Substrate Reagent to each well. 9. Incubate 30 min at RT. 10. Add 50 µL of Stop Solution to each well. 11. Read at 450 nm immediately.
Reagent Preparation:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. 2. Sample dilution: If your samples need to be diluted, 1x Assay Diluent (Item E) should be used for dilution of serum/plasma/culture supernatants/urine. Suggested dilution for normal serum/plasma: 300-3000 fold*. * Please note that levels of the target protein may vary between

	different specimens. Optimal dilution factors for each sample must be determined by the investigator. 3. Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µL 1x Assay Diluent (Item E) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 80 µL MMP-9 standard from the vial of tem C, into a tube with 586.7 µL 1x Assay Diluent Buffer to prepare a 6000 pg/mL stock standard solution. Pipette 400myl 1x Assay Diluent into each tube. Use the stock standard solution to produce a Dilution series . Mix each tube thoroughly before the next transfer. 1x Assay Diluent serves as the zero standard (0 pg/mL). 200 µL 80 µL standard +586.7 µL 200myl 200 µL 200 µL 200 µL 200 µL 6000 2000 666.7 222.2 74.07 24.69 8.23 0 pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL pg/mL 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 100-fold with 1x Assay Diluent and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 400-fold with 1x Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 30 µL of HRP-Streptavidin concentrate into a tube with 12 ml 1x Assay Diluent to prepare a 400-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.
Assay Procedure:	1. Bring all reagents and samples to room temperature (18 - 25 °C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µL of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 myl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µL of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

Application Details

	5. Discard the solution. Repeat the wash as in step 6. Add 100 µL of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 8. Add 100 µL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.
Calculation of Results:	Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. Typical Data: These standard curves are for demonstration only. A standard curve must be run with each assay. Assay Diluent Buffer Human MMP-9 concentration (pg/mL) 1 10 100 1000 10000 O D =4 50 n m 0.01 0.1 1 10 Sensitivity: The minimum detectable dose of MMP-9 is typically less than 10 pg/mL. Recovery: Recovery was determined by spiking various levels of human MMP-9 into normal human serum, plasma and cell culture media. Mean recoveries are as follows: Sample Type Average % Recovery Range (%) Serum 96.23 84-103 Plasma 94.64 83-102 Cell culture media 95.38 84-104 Linearity: Sample Type Serum Plasma Cell Culture Media 1:2 Average % of Expected 95 93 94 Range (%) 84-103 83-102 85-104 1:4 Average % of Expected 96 97 96 Range (%) 85-104 86-105 83-103 Reproducibility: Intra-Assay: CV<10 % Inter-Assay: CV<12 %
Assay Precision:	Intra-Assay: CV< 10 % Inter-Assay: CV< 12 %
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.
Expiry Date:	6 months

Publications

Product cited in:

Hanedan Onan, Baykan, Sezer, Narin, Mavili, Baykan, Uzum, Narin: "Evaluation of Cardiovascular Changes in Children with BAVs." in: **Pediatric cardiology**, Vol. 37, Issue 3, pp. 472-81, (2017) (PubMed).

Wu, Liu, Xie: "Osteopontin facilitates invasion in human trophoblastic cells via promoting matrix metalloproteinase-9 in vitro." in: **International journal of clinical and experimental pathology**, Vol. 8, Issue 11, pp. 14121-30, (2016) (PubMed).

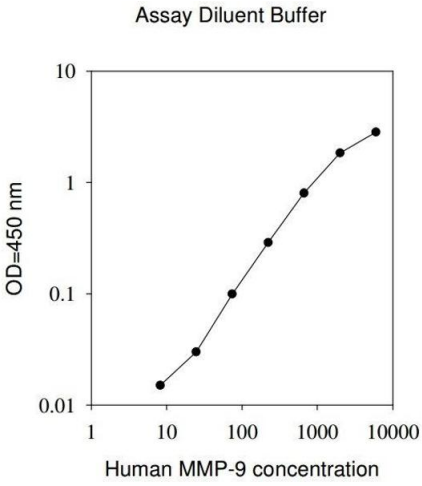
Devanarayanan, Nandeesha, Kattimani, Sarkar: "Relationship between matrix metalloproteinase-9 and oxidative stress in drug-free male schizophrenia: a case control study." in: **Clinical chemistry and laboratory medicine**, Vol. 54, Issue 3, pp. 447-52, (2016) (PubMed).

Prato, Khadjavi, Magnetto, Gulino, Rolfo, Todros, Cavalli, Guiot: "Effects of oxygen tension and dextran-shelled/2H,3H-decafluoropentane-cored oxygen-loaded nanodroplets on secretion of gelatinases and their inhibitors in term human placenta." in: **Bioscience, biotechnology, and biochemistry**, Vol. 80, Issue 3, pp. 466-72, (2016) (PubMed).

Wang, Song, Chen, Yuan, Xu, Zhang, Tan, Yang, Yu, Lv: "The Long-Term Influence of Tissue Inhibitor of Matrix Metalloproteinase-1 in Patients with Mild to Moderate Coronary Artery Lesions in a Chinese Population: A 7-Year Follow-Up Study." in: **Cardiology**, Vol. 132, Issue 3, pp. 151-8, (2016) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1.