Datasheet for ABIN5564622

MMP 9 ELISA Kit

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Overview

Quantity:	96 tests
Target:	MMP 9 (MMP9)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.39-25 ng/mL
Minimum Detection Limit:	0.39 ng/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax™ Human MMP-9 ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human MMP-9 in plasma, serum, urine, saliva, CSF, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human MMP-9 in approximately 4 hours. A polyclonal antibody specific for human MMP-9 has been pre-coated onto a 96-well microplate with removable strips. MMP-9 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for human MMP-9, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Brand:	AssayMax™
Sample Type:	Cell Culture Cells, Cerebrospinal Fluid, Plasma, Saliva, Serum, Urine
Analytical Method:	Quantitative

Product Details

Detection Method:	Colorimetric
Components:	Human MMP-9 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human MMP-9. Sealing Tapes: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay. Human MMP-9 Standard: Human MMP-9 in a buffered protein base (25 ng, lyophilized). Biotinylated Human MMP-9 Antibody (50x): A 50-fold concentrated biotinylated polyclonal antibody against human MMP-9 (120 l). MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (30 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml, 2 bottles). Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate (80 l). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 405 nm. Pipettes (1-20 µL, 20-200 µL, and multiple channel). Deionized or distilled reagent grade water Incubator (37 °C)

Target Details

Target:	MMP 9 (MMP9)
Alternative Name:	Matrix Metalloproteinase-9 (MMP-9) (MMP9 Products)
Background:	Matrix metalloproteinase-9 (MMP-9) is also known as 92 kDa type IV collagenase, 92- kDa gelatinase, gelatinase B, and type V collagenase. MMP-9 is a class of enzymes that belong to the zinc-metalloproteinases family involved in the degradation of the extracellular matrix. MMP-9 is synthesized as a preproenzyme of 707 amino-acid residues and secreted as an inactive proprotein which is activated when cleaved by extracellular proteinases. MMP-9 degrades type IV and V collagens into large C-terminal three quarter fragments and shorter N-terminal one quarter fragments. It also degrades fibronectin but not laminin or Pz-peptide. MMP-9 is involved in the breakdown of the extracellular matrix in normal physiological processes, such as embryonic development, reproduction and tissue remodeling, and in leukocyte migration. It plays roles in IL-8-induced mobilization of hematopoietic progenitor cells from bone marrow and bone osteoclastic resorption (1-2).
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

Plate:	Pre-coated
Protocol:	• Step 1. Add 50 µL of Standard or Sample per well. Incubate 2 hours. • Step 2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 1 hour. • Step 3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes. • Step 4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 25 minutes. • Step 5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water. Store for up to 30 days at 2-8 °C. Human MMP-9 Standard: Reconstitute the Human MMP-9 Standard (25 ng) with 1 mL of MIX Diluent to generate a 25 ng/mL standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (25 ng/mL) 2-fold with MIX Diluent to produce 12.5, 6.25, 3.125, 1.563, 0.781, and 0.391 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be frozen at -20 °C and used within 30 days. Standard Point Dilution [MMP-9] (ng/mL) P1 1 part Standard (25 ng/mL) 25 P2 1 part P1 + 1 part MIX Diluent 12.5 P3 1 part P2 + 1 part MIX Diluent 6.25 P4 1 part P3 + 1 part MIX Diluent 3.125 P5 1 part P4 + 1 part MIX Diluent 1.563 P6 1 part P5 + 1 part MIX Diluent 0.781 P7 1 part P6 + 1 part MIX Diluent 0.391 P8 MIX Diluent 0.0 5 Biotinylated Human MMP-9 Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with MIX Diluent. The undiluted antibody should be stored at -20 °C. Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent. The undiluted conjugate should be stored at -20 °C.
Sample Collection:	Platelet-Poor Plasma: Collect plasma using heparin as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. Additional centrifugation might be necessary for complete platelet removal. A 60-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 60-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3

	months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1x, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes. A 100-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20 °C or below for up to 3 months. Avoid repeated freeze-thaw cycles. CSF: Collect cerebrospinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. A 4-fold sample dilution is suggested into MIX Diluent, however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80 °C for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes at 4 °C to remove debris and collect supernatants. Samples can be stored at -20 °C or below. Avoid repeated freeze-thaw cycles. Refer to Sample Dilution Guidelines for further instruction. 4 Guidelines for Dilutions of 100-fold or Greater (for reference only, please follow the insert for specific dilution suggested) 100x 10000x A) 4 µL sample: 396 µ L buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL. A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL. 1000x 100000x A) 4 µL sample : 396 µL buffer (100x) B) 24 µL of A : 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL. A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) C) 24 µL of B : 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL.
Assay Procedure:	Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 50 l of Human MMP-9 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition. Wash five times with 200 l of Wash Buffer manually. Invert the plate each time and decant the contents, hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 l of Wash Buffer and then invert the plate, decanting the contents, hit 4-5 times on absorbent material to completely remove the liquid. Add 50 l of Biotinylated Human MMP-9 Antibody to each well. Gently tap plate to thoroughly coat the wells.

Application Details

	Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour. Wash the microplate as described above. Add 50 l of Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 l of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue color density develops. Add 50 l of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. 6 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	• Calculate the mean value of the duplicate or triplicate readings for each standard and sample. • To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. • Determine the unknown sample concentration from the standard curve and multiply the value by the dilution factor.
Restrictions:	For Research Use only

Handling

Handling Advice:	This product is for Research Use Only and is not intended for use in diagnostic procedures. 2 Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay. Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor. Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents. The Stop Solution is an acidic solution. The kit should not be used beyond the expiration date.
Storage:	4 °C,-20 °C
Storage Comment:	Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date. Store SP Conjugate and Biotinylated Antibody at -20°C. Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.

Handling

Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator. Diluent (1x) may be stored for up to 30 days at 2-8°C. Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent. 3

Images

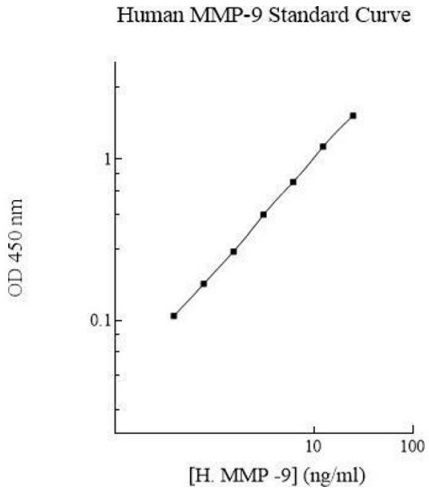

Image 1.