Datasheet for ABIN364941

MMP3 ELISA Kit

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Overview

Quantity:	96 tests
Target:	MMP3
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	0.156-10 ng/mL
Minimum Detection Limit:	0.156 ng/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of Human MMP-3. I concentrations in cell culture supernates, serum, plasma and other biological fluids. I
Sample Type:	Serum, Plasma, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Human MMP3.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	0.054 ng/mL
Components:	• Assay plate (12 × 8 coated Microwells)

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Product Details

- Standard (freeze dried)
- Biotin-antibody (100 × concentrate)
- HRP-avidin (100 × concentrate)
- Biotin-antibody Diluent
- HRP-avidin Diluent
- Sample Diluent
- Wash Buffer (25 × concentrate)
- TMB Substrate
- Stop Solution
- Adhesive Strip (for 96 wells)
- Instruction manual

Target Details

Target:	MMP3
Alternative Name:	Matrix metalloproteinase 3 (MMP-3) (MMP3 Products)
Background:	Synonyms: CHDS6, MGC126102, MGC126103, MGC126104, MMP-3, SL-1, STMY, STMY1, STR1, matrix metalloproteinase 3|matrix metalloproteinase 3 (stromelysin 1, progelatinase)|proteoglycanase|transin-1
HGNC:	7162
UniProt:	P08254

Application Details

Application Notes:

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
- Grossly hemolyzed samples are not suitable for use in this assay.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not

be recognized by this supplier's products.
- Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Comment:	Detection wavelength: 450 nm Information on standard material: Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative. Information on reagents: In most cases the stop solution provided is 1 N H2SO4. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA. Information on antibodies: The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A
Sample Volume:	100 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	Antibody specific for MMP3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MMP3 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for MMP3 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of MMP3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

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Application Details

Reagent Preparation:

- **Biotin-antibody (1×)** - Centrifuge the vial before opening.
  Biotin-antibody requires a 100-fold dilution. The suggested dilution is 10μL of Biotin-antibody + 990μL of Biotin-antibody Diluent.
- **HRP-avidin (1×)** - Centrifuge the vial before opening.
  HRP-avidin requires a 100-fold dilution. The suggested dilution is 10μL of HRP-avidin + 990μL of HRP-avidin Diluent.
- **Wash Buffer (1×)** - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×).
- **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s.
  Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 200pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.
  Pipette 250μL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (200pg/mL). Sample Diluent serves as the zero standard (0ng/mL).

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25°C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μL when pipetting.
- It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.

Assay Precision:

Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision.

Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision.

- Intra-assay: CV% less than 8%
- Inter-assay: CV% less than 10%

Restrictions:

For Research Use only

Handling

Precaution of Use:	The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.
Handling Advice:	• The kit should not be used beyond the expiration date on the kit label. • Do not mix or substitute reagents with those from other lots or sources. • If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay. • Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding. • This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
Expiry Date:	6 months

Publications

Product cited in:	Li, Wang, An, Liang, Zhu, Lian, Niu, Guo, Tian: "Bone marrow mesenchymal stem cells attenuate silica-induced pulmonary fibrosis via paracrine mechanisms." in: **Toxicology letters**, Vol. 270, pp. 96-107, (2017) (PubMed).

Successfully validated (ELISA (ELISA))

by CGIBD Advanced Analytics Core

Report Number: 029765

Date: Jul 19 2014

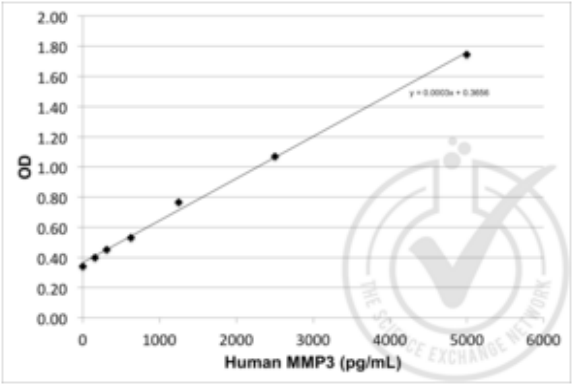
Lot Number:	V25184071
Method validated:	ELISA (ELISA)
Positive Control:	Human serum - expression is ~16.5 ng/mL
Negative Control:	Goat serum (non-reactive species)
Notes:	Target protein was detected in the positive control sample and not in the negative control sample as expected.
Controls:	• Positive control: Human serum (Sigma Aldrich, Cat# H6914-20ML, Lot# SLBK2170V) • Negative control: Goat serum (Sigma Aldrich, Cat# G9023-10ML, Lot# SLBH2670V)
Protocol:	• 1. All reagents were brought up to room temperature for 30 minutes prior to use. The 1x Wash Buffer was prepared by adding 20 mL of 25x Wash Buffer Concentrate to 480 mL of distilled/deionized water and mixing thoroughly. • 2. The vial of Standard was reconstituted with 1 mL of Sample Diluent, mixed, and allowed to sit for 15 minutes with gentle agitation. • 3. The standard curve was prepared by creating a 2-fold dilution series of seven standards (including the original undiluted vial) using Sample Diluent. Sample Diluent alone served as the 0 pg/mL standard. • 4. The assay plate was removed from the foil pouch and 100 µL of each standard and sample were added to the appropriate wells, in triplicate. The plate was covered with the adhesive strip provided and incubated for 2 hours at 37 °C. • 5. Approximately 10 minutes before the incubation ended, a 1x Biotin-antibody solution was prepared by diluting 60 µL of 100x Biotin-antibody into 5940 µL of Biotin-antibody Diluent. • 6. The liquid from each well was removed. • 7. 100 µL of 1x Biotin-antibody solution was added to each well, and the plate was covered with a new adhesive strip, and incubated for 1 hour at 37 °C. • 8. Approximately 10 minutes before the incubation ended, a 1x HRP-avidin solution was prepared by diluting 60 µL of 100x HRP-avidin into 5940 µL of HRP-avidin Diluent. • 9. Each well was aspirated and washed, repeating the process two times for a total of three washes. Each well was washed by filling each well with 1x Wash Buffer and letting it stand for 2 minutes. After the last wash, remaining Wash Buffer was removed and the plate was inverted and blotted against clean, absorbent paper towels. • 10. 100 µL of 1x HRP-avidin solution was added to each well, the plate was covered with a

new adhesive strip, and incubated for 1 hour at 37 °C.

- 11. The aspiration/wash procedure from Step 9 was repeated for an additional 5 washes.
- 12. 90 µL of TMB Substrate was added to each well. The plate was protected from light and incubated for 15-30 minutes at 37 °C, with periodic checking to prevent overdevelopment.
- 13. 50 µL of Stop Solution was added to each well and mixed thoroughly. The optical density (OD) of each well was measured within 5 minutes using a microplate reader set to 450 nm.
- 14. A standard curve was generated by plotting the OD value for each standard on the y-axis against the concentration on the x-axis using Excel. A line of best fit through the points on the graph was used to generate the equation x = (y-0.3656) / 0.0003.
- 15. The equation was used to calculate MMP3 concentrations of the samples based on their average OD values.

Experimental Notes: Linearity was poor between positive control samples at different dilutions. This may be indicative of possible interference/inhibition in normal serum.

Images for Validation report #029765



Validation image no. 1 for Matrix Metallopeptidase 3 (Stromelysin 1, Progelatinase) (MMP3) ELISA Kit (ABIN364941)

Figure 1: MMP3 standard curve graph.

	1	2	3	Average OD	MMP3 (pg/mL)
Positive control human serum	0.98	0.96	0.90	0.95	1936.89
Positive control human serum 1:2	1.23	1.31	1.09	1.21	2619.11
Positive control human serum 1:5	1.03	1.08	0.89	1.00	2111.33
Positive control human serum 1:10	0.81	0.81	0.66	0.76	1312.44
Positive control human serum 1:20	0.55	0.55	0.48	0.53	535.78
Positive control human serum 1:40	0.33	0.35	0.29	0.32	-143.11
Positive control human serum 1:50	0.29	0.28	0.26	0.28	-302.00
Negative control goat serum	0.03	0.03	0.03	0.03	-1117.56

Validation image no. 2 for Matrix Metallopeptidase 3 (Stromelysin 1, Progelatinase) (MMP3) ELISA Kit (ABIN364941)

Figure 2: Table of absorbance readings (OD 450 nm) for positive (human serum) and negative (goat serum) control samples. Value for Average Reading is derived from the average of three readings (OD 450nm). An equation x = (y-0.3656) / 0.0003 was generated from the standard curve and used to calculate MMP3 concentrations shown in the Table.