colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human MMP-3 in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00094
Product Name	MMP-3 ELISA Kit
Species cross-reactivity	Human MMP-3
Range (calibration Range)	0.4 - 96 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**4314** (Human)
SwissProt	**P08254** (Human)

kit components & storage

Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 96 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3-ef- 30 mL/bottle; For serum, plasma samples	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 4-eg- 30 mL/bottle; For cell culture supernatants	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	3 pieces	

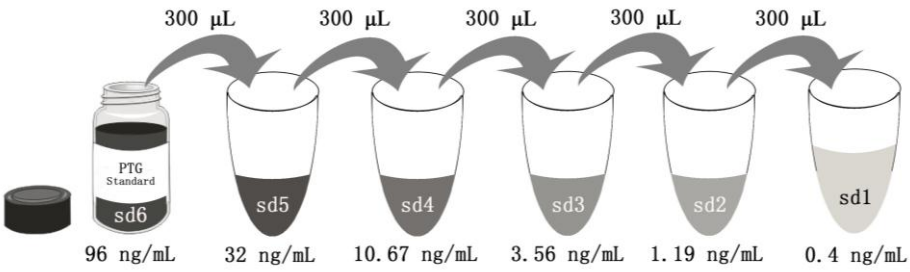
NB: Do not use the kit after the expiration date.

Sample Diluent PT 3-ef is for Standard and serum, plasma samples.

Sample Diluent PT 4-eg is for standard, cell culture supernatants.

Detection Diluent is for Detection antibody and HRP-conjugated antibody.

*Add **1 mL** Sample Diluent PT 3-ef or PT 4-eg in Standard, This reconstitution gives a stock solution of 96 ng/mL.



Add # µL of Standard diluted in the previous step	—	300 µL	300 µL	300 µL	300 µL	300 µL
# µL of Sample Diluent PT 3-ef or PT 4-eg	**1000 µL**	600 µL	600 µL	600 µL	600 µL	600 µL
	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00094 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The MMP-3 ELISA kit is to be used to detect and quantify protein levels of endogenous MMP-3. The assay recognizes human MMP-3 . A polyclonal antibody specific for MMP-3 has been pre-coated onto the microwells. The MMP-3 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for MMP-3 is added to detect the captured MMP-3 protein. For signal development, horseradish peroxidase (HRP)-conjugated antibody is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

background

Matrix metalloproteinases (MMPs) play a critically important role in extracellular matrix remodeling and have been implicated in a number of key normal and pathologic processes.These proteases have come to represent important therapeutic and diagnostic targets for the treatment and detection of human cancers. MMP-3 activate procollagenase via two pathways: slow direct activation and rapid activation in conjunction with tissue or plasma proteinases. The pro-MMP3 (60 kDa) and the active MMP3 (47 kDa) can be detected through western blot.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:2, 1:4 is suggested according to the individual samples.

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safety notes

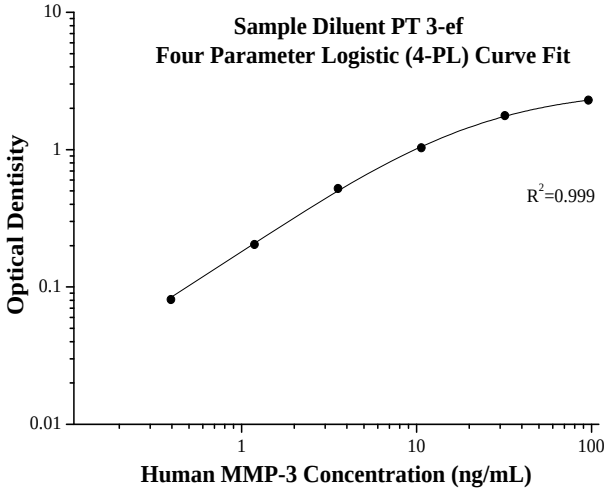
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	**120** min	4 times	Cover Wells
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells
4	TMB Substrate	100 µL	15-30 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.064 0.062	0.063	—
0.4	0.15 0.137	0.144	0.081
1.19	0.274 0.26	0.267	0.204
3.56	0.575 0.593	0.584	0.521
10.67	1.094 1.092	1.093	1.03
32	1.809 1.842	1.826	1.763
96	2.342 2.359	2.351	2.288

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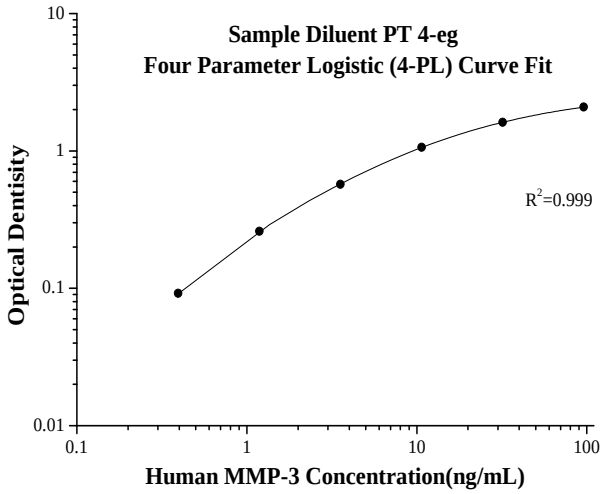

(ng/mL)	O.D	Average	Corrected
0	0.054 0.053	0.054	—
0.4	0.147 0.144	0.146	0.092
1.19	0.304 0.323	0.314	0.260
3.56	0.623 0.629	0.626	0.572
10.67	1.123 1.112	1.118	1.064
32	1.681 1.653	1.667	1.613
96	2.195 2.081	2.138	2.084

precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (ng/mL)	3.8	13.3	51.8	3.5	13.5	50.2
SD	0.32	1.16	4.69	0.19	0.91	4.98
CV%	8.3	8.7	9.1	5.4	6.7	9.9

recovery

The recovery of MMP-3 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated. (The plasma samples were initially diluted 1:2)

Sample Type		Average % of Expected	Range(%)
Citrate plasma	1:2	84	76-101
	1:4	86	77-97
Cell culture supernatants	1:2	84	75-92
	1:4	85	70-111

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sample value

Serum Samples from healthy volunteers were evaluated for MMP-3 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=30)	1.06	0.763-1.426

sensitivity

The minimum detectable dose of human MMP-3 is 0.01 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of MMP-3 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.
(The plasma samples were initially diluted 1:2)

		Citrate plasma (Sample Diluent PT 3-ef)	Cell culture supernatants (Sample Diluent PT 4-eg)
1:2	Average% of Expected	70	78
	Range(%)	62-85	70-86
1:4	Average% of Expected	76	93
	Range(%)	72-77	84-102
1:8	Average% of Expected	91	93
	Range(%)	78-108	91-96
1:16	Average% of Expected	88	88
	Range(%)	82-108	84-91

references

1. Roy R. et al. (2009) J Clin Oncol. 27:5287-97.
2. Suzuki K. et al. (1990) Biochemistry. 29:10261-70.
3. O'Brien M. et al. (2007) Mol Hum Reprod. 13:655-61.
4. Itoh Y. et al .(2002) Essays Biochem. 38:21-36.