

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human ECM1 in serum, plasma and cell culture supernatants.

general information

Catalogue Number	KE00141
Product Name	ECM1 ELISA Kit
Species cross-reactivity	Human ECM1
Range (calibration Range)	78.1 – 5000 pg/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	1893 (Human)
SwissProt	Q16610 (Human)

kit components & storage

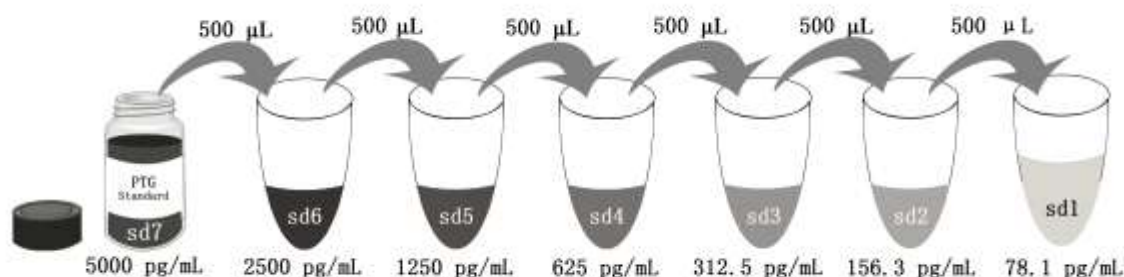
Microplate - antibody coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard – 10000 pg/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, HRP-conjugated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1 - 30 mL/bottle	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 1 is for standard and samples

Detection Diluent is for Detection antibody.

*Add 2 mL Sample Diluent PT 1 in Standard. This reconstitution gives a stock solution of 5000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00141 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The ECM1 ELISA kit is to be used to detect and quantify protein levels of endogenous ECM1. The assay recognizes human ECM1. An antibody specific for ECM1 has been pre-coated onto the microwells. The ECM1 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another horseradish peroxidase (HRP)-conjugated antibody specific for ECM1 is added to detect the captured ECM1 protein. For signal development, 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 with the correction wavelength set at 630 nm .

background

Extracellular matrix protein 1 (ECM1) is a glycoprotein involved in a number of biological processes such as bone formation, skin differentiation, cell proliferation, and promotes angiostasis. ECM1 is expressed in breast cancer and could participate in cell migration and angiogenesis. Pathologically, ECM1 contributes to the formation and metastasis of several types of cancer including breast, thyroid and hepatocellular cancers. It can be detected in human serum. ECM1 protein is a possible trigger for angiogenesis, tumor progression and malignancies.

sample preparation

The serum or plasma, cell culture supernatants may require proper dilution to fall within the range of the assay. 1:3,000 or 1:6,000 dilution is recommended for healthy human serum or plasma. 1:2 dilution is recommended for cell culture supernatants.

safety notes

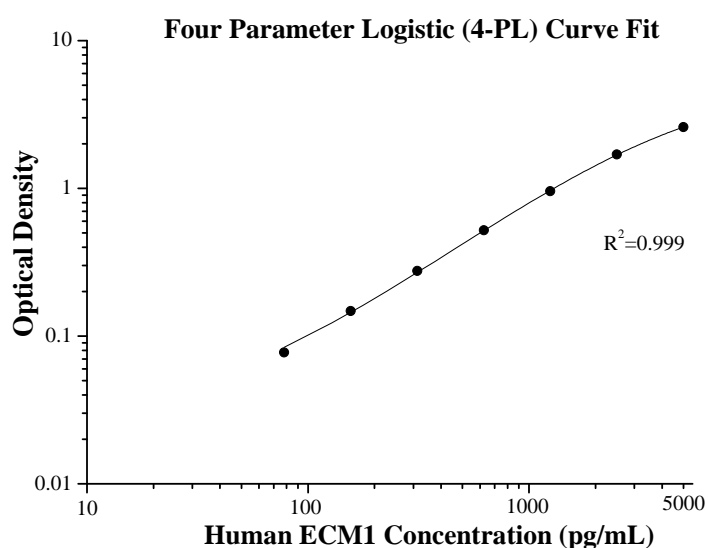
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	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.



(pg/mL)	O.D	Average	Corrected
0	0.059	0.061	—
	0.062		
78.1	0.139	0.138	0.078
	0.137		
156.3	0.202	0.208	0.148
	0.214		
312.5	0.335	0.337	0.276
	0.338		
625	0.575	0.581	0.521
	0.587		
1250	0.994	1.018	0.958
	1.042		
2500	1.748	1.753	1.693
	1.758		
5000	2.662	2.661	2.6
	2.659		

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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	24	24	24
Mean (pg/mL)	2,305.5	615.7	116.9	2,070.5	572.8	152.8
SD	46.3	24.9	9.4	108.7	29.9	15.9
CV%	2.0	4.1	8.0	5.3	5.2	10.4

recovery

The recovery of ECM1 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average % of Expected	Range (%)
Human serum	1:3,000	101	81-114
	1:6,000	97	84-111
Cell culture supernatants	1:2	88	71-95
	1:4	83	72-96

sample values

Serum samples from healthy volunteers were evaluated for ECM1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean(ng/mL)	Rang (ng/mL)
Human serum (n=24)	2,468	273-4,687

sensitivity

The minimum detectable dose of human ECM1 is 0.1 pg/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, human serum samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The serum samples were initially diluted 1:800)

To assess the linearity of the assay, cell culture supernatants were spiked with high concentrations of ECM1 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatants
1:2	Average% of Expected	95	101
	Range (%)	93-96	100-101
1:4	Average% of Expected	96	101
	Range (%)	88-104	100-102
1:8	Average% of Expected	94	99
	Range (%)	90-98	98-100
1:16	Average% of Expected	96	98
	Range (%)	85-105	97-99

references

1. Chen H. et al. (2010). Med Oncol. 1:S318-25.
2. López-Marure R. et al. (2011). Eur J Pharmacol. 660(2-3):268-74.
3. Lee KM. et al. (2015). Oncogene. 34(50):6055-65.