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colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human ADAM12 in serum, plasma, cell culture supernatants and urine.

general information

Catalogue Number	KE00058
Product Name	ADAM12 ELISA Kit
Species cross-reactivity	Human ADAM12
Range (calibration Range)	0.78 - 50 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	**8038** (Human)
SwissProt	**O43184** (Human)

kit components & storage

Microplate - antibody coated 96-well Microplate (8 wells × 12 strips)	1 plate	Store at 2-8°C for six months
Standard -100 ng/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-ef - 30 mL/bottle. For serum and plasma samples	1 bottle	Store at 2-8°C for six months
Sample Diluent PT 1-eg - 30 mL/bottle. For cell culture supernatants and urine samples	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	

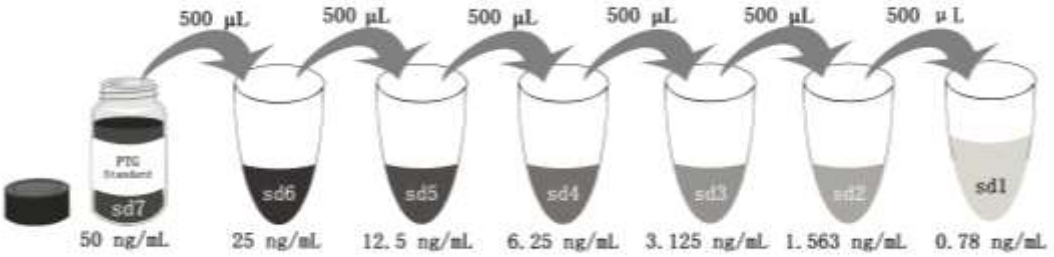
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ef is for standard, serum and plasma samples.

Sample Diluent PT 1-eg is for standard, cell culture supernatants and urine samples.

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

*Add 2 mL Sample Diluent PT 1-ef or PT 1-eg in standard. This reconstitution gives a stock solution of 50 ng/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-ef or PT 1-eg	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00058 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The ADAM12 ELISA kit is to be used to detect and quantify protein levels of endogenous ADAM12. The assay recognizes human ADAM12. An antibody specific for ADAM12 has been pre-coated onto the microwells. The ADAM12 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for ADAM12 is added to detect the captured ADAM12 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 with the correction wavelength set at 630 nm.

background

ADAM12, also named as MLTN and Meltrin-alpha, is involved in skeletal muscle regeneration, specifically at the onset of cell fusion. It is also involved in macrophage-derived giant cells (MGC) and osteoclast formation from mononuclear precursors. ADAM12 is expressed in human malignant tumors. It is involved in the regulation of growth factor activities and integrin functions, leading to promotion of cell growth and invasion, although the precise mechanisms of these are not clear at the present time. ADAM12's ability to degrade extracellular matrix components likely allows it to detach cancer cells from the basement membrane and assist them on their route to metastasis. But the protein's role not just as a biomarker of breast cancer but as a gateway to cancer cell migration is only now being understood.

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

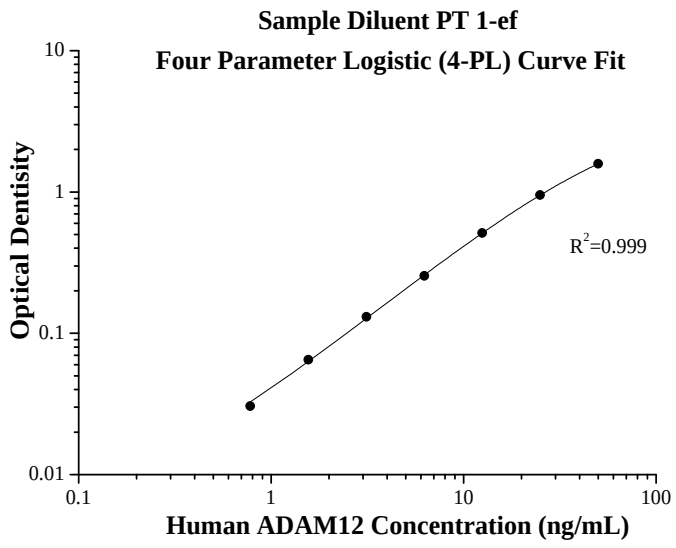
This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	**120 min**	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 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	0.068 0.061	0.0645	—
0.78	0.098 0.092	0.095	0.0305
1.56	0.129 0.13	0.1295	0.065
3.13	0.198 0.192	0.195	0.1305
6.25	0.329 0.31	0.3195	0.255
12.5	0.578 0.578	0.578	0.5135
25	1.012 1.016	1.014	0.9495
50	1.681 1.612	1.6465	1.582

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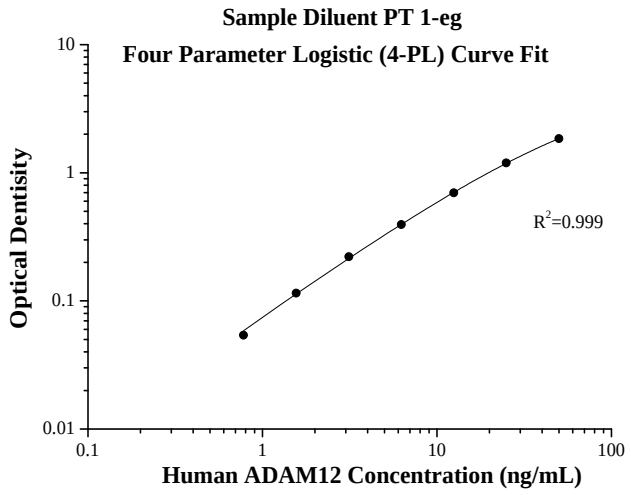

(ng/mL)	O.D	Average	Corrected
0	0.055 0.06	0.0575	—
0.78	0.11 0.113	0.1115	0.054
1.56	0.174 0.171	0.1725	0.115
3.13	0.28 0.277	0.2785	0.221
6.25	0.461 0.443	0.452	0.3945
12.5	0.757 0.753	0.755	0.6975
25	1.215 1.282	1.2485	1.191
50	1.943 1.867	1.905	1.8475

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)	26.8	10.6	2.6	25.5	10.3	2.4
SD	1.26	0.45	0.14	0.99	0.59	0.12
CV%	4.7	4.3	5.5	3.9	5.7	4.8

recovery

The recovery of ADAM12 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 plasma	1:4	93	77-120
	1:8	94	76-128
Cell culture supernatants	1:2	103	92-116
	1:4	92	81-108
Urine	1:1	113	89-123
	1:2	92	75-108

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sample value

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human ADAM12 in this assay. All samples measured less than the lowest standard, 0.78 ng/mL. No medical histories were available for the donors used in this study.

sensitivity

The minimum detectable dose of human ADAM12 is 0.63 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 ADAM12 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.
(The samples were initially diluted 1:3)

		Human plasma (Sample Diluent PT 1-ef)	Cell culture supernatants (Sample Diluent PT 1-eg)	Urine (Sample Diluent PT 1-eg)
1:2	Average% of Expected	91	112	125
	Range (%)	77-105	98-126	113-137
1:4	Average% of Expected	92	108	107
	Range (%)	86-97	104-112	102-112
1:8	Average% of Expected	98	102	99
	Range (%)	94-101	99-105	93-105
1:16	Average% of Expected	101	97	103
	Range (%)	94-108	94-101	99-107

references

1. Mochizuki S1, Okada Y. ADAMs in cancer cell proliferation and progression. Cancer Sci. 2007 May;98(5):621-8.
2. Georges S, et al. A Disintegrin And Metalloproteinase 12 produced by tumour cells accelerates osteosarcoma tumour progression and associated osteolysis. Eur J Cancer. 2013 Jun;49(9):2253-63.
3. Stautz D, et al. Functional analysis of a breast cancer-associated mutation in the intracellular domain of the metalloprotease ADAM12. PLoS One. 2012;7(5):e37628.