

## colorimetric sandwich ELISA kit datasheet

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

### general information

Catalogue Number	KE00065
Product Name	MCAM ELISA Kit
Species cross-reactivity	Human MCAM
Range (calibration Range)	62.5 - 4000 ng/mL
Tested applications	Quantification ELISA

### database links

Entrez Gene	4162 (Human)
SwissProt	P43121 (Human)

### kit components & storage

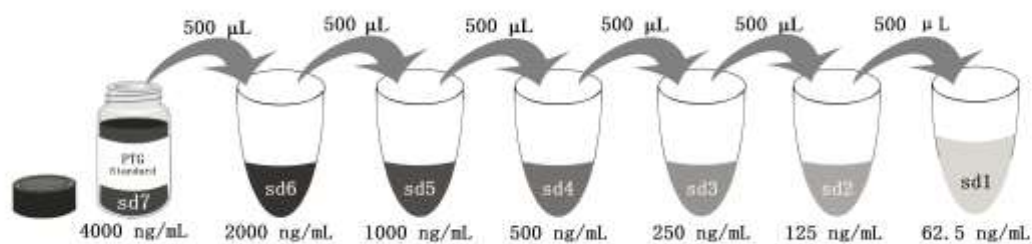
Microplate - antibody coated 96-well Microplate (8 wells × 12 strips)	1 plate	Store at -20°C for six months
Standard - 4000 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection Antibody (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated (100X) - 120 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 3-ef- 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 3-ef is for standard, serum, plasma and cell culture supernatants samples.

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

\*Add 1 mL Sample Diluent PT 3-ef in Standard. This reconstitution gives a stock solution of 4000 ng/mL.



Add # $\mu\text{L}$ of Standard diluted in the previous step	—	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$
# $\mu\text{L}$ of Sample Diluent PT 3-ef	1000 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$	500 $\mu\text{L}$
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

## product description

KE00065 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The MCAM ELISA kit is to be used to detect and quantify protein levels of endogenous MCAM. The assay recognizes human MCAM. An antibody specific for MCAM has been pre-coated onto the microwells. The MCAM protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody specific for MCAM is added to detect the captured MCAM 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

MCAM, also known as melanoma cell adhesion molecule (CD146) or MUC18, originally identified as a biomarker of melanoma progression, is a transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily. Structurally, it consists of five Ig domains, a transmembrane domain, and a cytoplasmic region. A soluble form of CD146 has also been reported. In normal adult tissue, CD146 is primarily expressed by vascular endothelium and smooth muscle. CD146 is a key cell adhesion protein in vascular endothelial cell activity and angiogenesis, and has been used as marker of circulating endothelium cells (CECs).

## sample preparation

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

## safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.

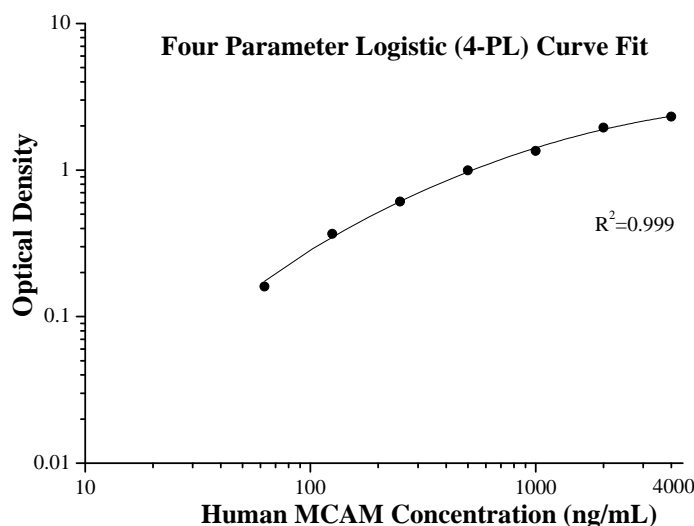
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	<b>120 min</b>	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	0.072	0.071	-
	0.07		
62.5	0.253	0.232	0.161
	0.21		
125	0.441	0.438	0.367
	0.435		
250	0.664	0.680	0.609
	0.695		
500	1.035	1.066	0.995
	1.096		
1000	1.367	1.424	1.353
	1.48		
2000	1.99	2.02	1.949
	2.05		
4000	2.349	2.385	2.314
	2.42		

## 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 (ng/mL)	2,643.0	487.4	124.3	1,647.6	344.9	82.4
SD	252.8	40.3	7.3	156.9	27.6	8.1
CV%	9.6	8.3	5.9	9.5	8.0	9.8

## recovery

The recovery of MCAM 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	1:16	88	79-121
	1:32	95	82-118
Cell culture supernatants	1:4	96	82-125
	1:8	94	80-112

## sample values

Twenty-four serum and plasma samples from healthy volunteers were evaluated for human MCAM in this assay.

Sample Type	Mean of Detectable (ng/mL)	Range (ng/mL)
Human serum (n=24)	422	114-2,198

## sensitivity

The minimum detectable dose of human MCAM is 3.8 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 MCAM in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

		Human plasma	Cell culture supernatants
		1:4	Average% of Expected
	Range (%)	83-92	83-109
1:8	Average% of Expected	93	106
	Range (%)	82-109	100-117
1:16	Average% of Expected	96	113
	Range (%)	82-114	110-115
1:32	Average% of Expected	94	100
	Range (%)	82-113	84-111

## references

1. Sers C, et al. Genomic organization of the melanoma-associated glycoprotein MUC18: implications for the evolution of the immunoglobulin domains. *Proc Natl Acad Sci U S A*. 90(18):8514-8 (1993).
2. Ouhtit A, et al. Towards understanding the mode of action of the multifaceted cell adhesion receptor CD146. *Biochim Biophys Acta*. 1795(2):130-6 (2009).
3. Bardin N, et al. CD146: biosynthesis and production of a soluble form in human cultured endothelial cells. *421(1):12-4 (1998)*.
4. Bardin N, et al. CD146 and its soluble form regulate monocyte transendothelial migration. *Arterioscler Thromb Vasc Biol*. 29(5):746-53 (2009).