

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human CD31 in serum and plasma.

general information

Catalogue Number	KE00041
Product Name	CD31 ELISA Kit
Species cross-reactivity	Human CD31
Range (calibration Range)	0.25 - 16 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	5175 (Human)
SwissProt	P16284 (Human)

kit components & storage

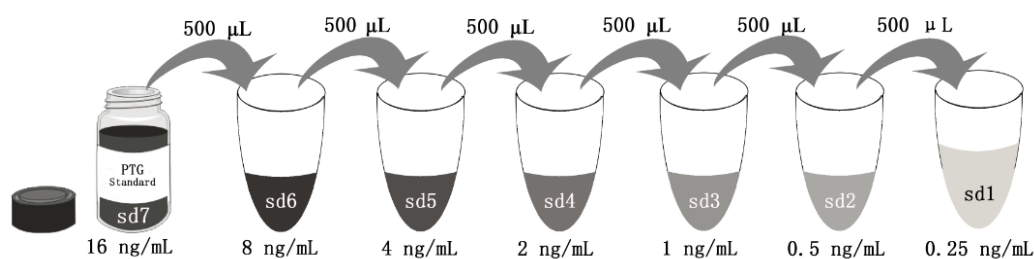
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 32 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-e - 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
Substrate, Tetramethylbenzidine (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-e is for Standard and Samples.

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

*Add 2 mL Sample Diluent PT 1-e in Standard, This reconstitution gives a stock solution of 16 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-e	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00041 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CD31 ELISA kit is to be used to detect and quantify protein levels of endogenous CD31. The assay recognizes human CD31. A polyclonal antibody specific for CD31 has been pre-coated onto the microwells. The CD31 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for CD31 is added to detect the captured CD31 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

Platelet endothelial cell adhesion molecule-1 (PECAM-1, CD31) is a member of the immunoglobulin gene superfamily of cell adhesion molecules. It is highly expressed on the surface of the endothelium, making up a large portion of its intracellular junctions. CD31 is also present on the surface of hematopoietic cells and immune cells including platelets, monocytes, neutrophils, natural killer cells, megakaryocytes and some types of T-cell. As well as its role in cell-cell adhesion, CD31 functions as a signaling receptor, and is involved in important physiological events such as nitric oxide production, regulation of T-cell immunity and tolerance, leukocyte transendothelial migration and inflammation and angiogenesis. Soluble form of CD31 is present in human plasma, resulting from alternative splicing of the transmembrane domain.

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.

safety notes

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

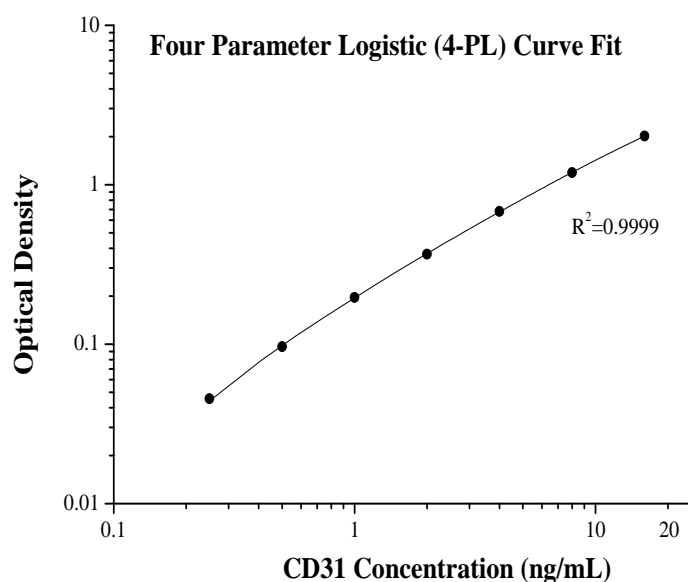
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 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	0.052	0.0515	—
	0.051		
0.25	0.097	0.097	0.0455
	0.097		
0.5	0.15	0.148	0.0965
	0.146		
1	0.242	0.2475	0.196
	0.253		
2	0.416	0.4185	0.367
	0.421		
4	0.731	0.7325	0.681
	0.734		
8	1.278	1.2445	1.193
	1.211		
16	2.099	2.071	2.0195
	2.043		

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)	15758.5	3819.4	742.1	14706.8	3729.3	89.0
SD	909.3	218.9	37.1	713.2	231.0	80.6
CV%	5.8	5.7	5.0	4.8	6.2	9.1

recovery

The recovery of CD31 spiked to three different levels in four samples throughout the range of the assay in human plasma averaged 96%, ranging from 87%-102%.

sensitivity

The minimum detectable dose of human CD31 is 0.09 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 CD31 in human plasma and diluted with the appropriate **Sample Diluent PT 1-e** to produce samples with values within the dynamic range of the assay. (The samples were initially diluted 1:1)

		Citrate plasma
1:2	Average% of Expected	86
	Range(%)	81-91
1:4	Average% of Expected	93
	Range(%)	90-98
1:8	Average% of Expected	93
	Range(%)	88-100
1:16	Average% of Expected	97
	Range(%)	86-111

references

1. Newman PJ, *et al.* The biology of PECAM-1. *J Clin Invest.* 99(1):3-8 (1997).
2. McCormick ME, *et al.* Platelet-endothelial cell adhesion molecule-1 regulates endothelial NO synthase activity and localization through signal transducers and activators of transcription 3-dependent NOSTRIN expression. *Arterioscler Thromb Vasc Biol.* 31(3):643-9 (2011).
3. Woodfin A, *et al.* PECAM-1: a multi-functional molecule in inflammation and vascular biology. *Arterioscler Thromb Vasc Biol.* 27(12):2514-23 (2007).
4. Goldberger A, *et al.* Biosynthesis and processing of the cell adhesion molecule PECAM-1 includes production of a soluble form. *J Biol Chem.* 269(25):17183-91 (1994).