

colorimetric sandwich ELISA kit datasheet

For the quantitative detection of human CD71 in serum and plasma.

general information

Catalogue Number	KE00081
Product Name	CD71 ELISA Kit
Species cross-reactivity	Human CD71
Range (calibration Range)	1.56 - 100 ng/mL
Tested applications	Quantification ELISA

database links

Entrez Gene	7037 (Human)
SwissProt	P02786 (Human)

kit components & storage

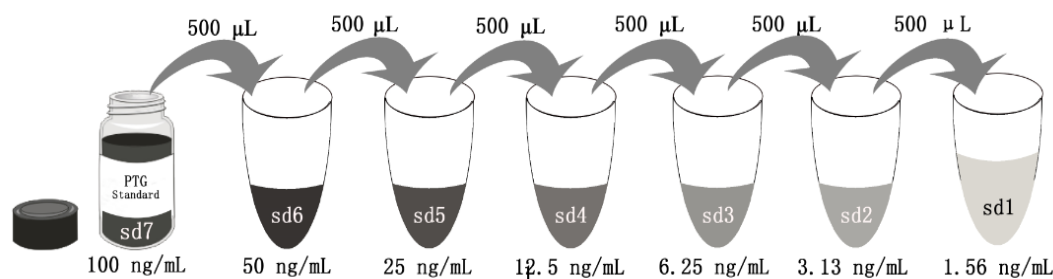
Microplate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Store at -20°C for six months
Standard - 200 ng/bottle; lyophilized*	2 bottles	Store at -20°C for six months
Detection antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
HRP-conjugated antibody (100X) - 150 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 1-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 1-ef is for Standard, serum and plasma samples.

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

*Add 2 mL Sample Diluent PT 1-ef in Standard, This reconstitution gives a stock solution of 100 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	2000 μL	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

product description

KE00081 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CD71 ELISA kit is to be used to detect and quantify protein levels of endogenous CD71. The assay recognizes human CD71. A polyclonal antibody specific for CD71 has been pre-coated onto the microwells. The CD71 protein in samples is captured by the coated antibody after incubation. Following extensive washing, a monoclonal antibody specific for CD71 is added to detect the captured CD71 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

CD71, also known as transferrin receptor protein 1 (TfR1), is a transmembrane glycoprotein which mediates cellular uptake of iron from transferrin, a plasma protein which transports iron in the circulation. CD71 is a homodimer of two identical transmembrane subunits linked by two disulfide bonds. CD71 is almost ubiquitously expressed, with highest expression levels on some cells and tissues, including immature erythroid cells, placental tissue, and rapidly dividing cells. Soluble CD71 present in the circulation is the truncated form of the cell surface receptor. The concentration of serum CD71 has been suggested to provide a sensitive measure of iron depletion.

sample preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. A range of dilutions like 1:10, 1:20 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.

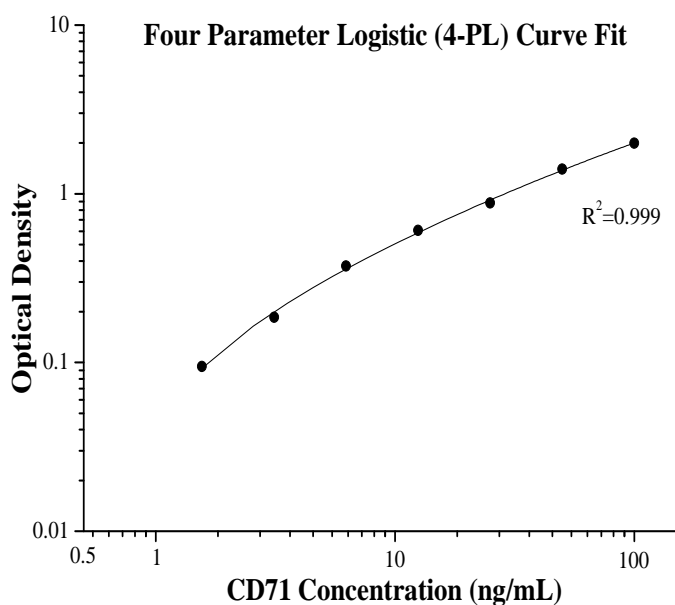
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

assay procedure summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	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	15-20 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	0.073	0.0725	—
	0.072		
1.56	0.158	0.167	0.0945
	0.176		
3.13	0.249	0.258	0.1855
	0.267		
6.25	0.407	0.4455	0.373
	0.484		
12.5	0.657	0.6785	0.606
	0.7		
25	0.95	0.954	0.8815
	0.958		
50	1.511	1.473	1.4005
	1.435		
100	2.053	2.07	1.9975
	2.087		

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)	99.5	19.3	3.8	80	17.8	4.9
SD	5.2	1.2	0.3	7.1	1	0.4
CV%	5.2	6.3	8.4	8.9	5.4	8.3

recovery

The recovery of TF 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(%)
Citrate plasma	1:10	88	83-94
	1:20	96	90-101

sensitivity

The minimum detectable dose of human CD71 is 0.2 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 CD71 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 1:5)

		Citrate plasma
1:2	Average% of Expected	108
	Range(%)	95-121
1:4	Average% of Expected	102
	Range(%)	96-108
1:8	Average% of Expected	105
	Range(%)	97-116
1:16	Average% of Expected	103
	Range(%)	93-114

references

1. Ponka P, et al. The transferrin receptor: role in health and disease. *Int J Biochem Cell Biol.* 31(10):1111-37 (1999).
2. Punnonen K, et al. Serum transferrin receptor and its ratio to serum ferritin in the diagnosis of iron deficiency. *Blood.* 89(3):1052-7 (1997).
3. Speeckaert MM, et al. Biological and clinical aspects of soluble transferrin receptor. *Crit Rev Clin Lab Sci.* 47(5-6):213-28 (2010).