Human CD22 Sandwich ELISA Kit Datasheet

For the quantitative detection of Human CD22 concentrations in serum, plasma, cell culture supernatants and cell lysates.

General Information

Catalogue Number	KE00131
Product Name	Human CD22 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	125-8000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	933
SwissProt	P20273

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 16000 pg/bottle; lyophilized*	2 bottles	
Detection antibody (100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 1 - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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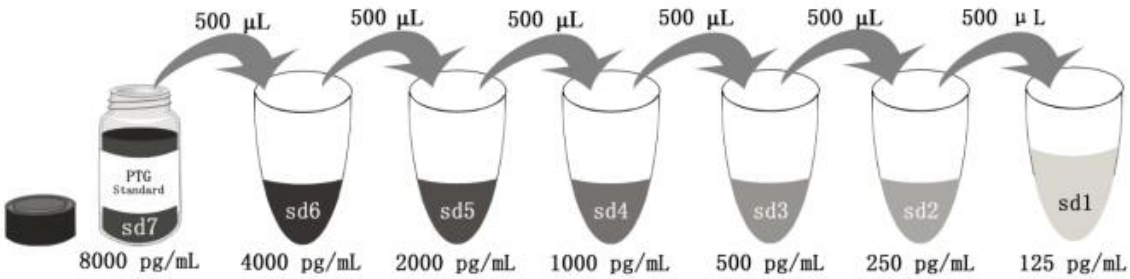
NB: Do not use the kit after the expiration date.
Sample Diluent PT 1 is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **2 mL** Sample Diluent PT 1 in protein standard. This reconstitution gives a stock solution of 8000 pg/mL.



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

KE00131 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The CD22 ELISA kit is to be used to detect and quantify protein levels of endogenous CD22. The assay recognizes human CD22. An antibody specific for CD22 has been pre-coated onto the microwells. The CD22 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for human CD22 is added to detect the captured human CD22 protein. For signal development, Streptavidin-HRP 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 450 nm with the correction wavelength set at 630 nm.

Background

CD22, also known as Siglec-2 (sialic acid binding Ig-like lectin 2) or BL-CAM (B-lymphocyte cell adhesion molecule), is a 130-140 kDa, B-cell restricted, type I transmembrane glycoprotein belonging to the immunoglobulin gene superfamily. The expression of CD22 is developmentally regulated. It is expressed at low levels in the cytoplasm of pro-B and pre-B cells and present on the cell surface only at mature stages of B-cell differentiation. Cell surface expression is lost during terminal differentiation into plasma cell and after B-cell activation. CD22 is an inhibitory receptor for B-cell receptor (BCR) signalling, preferentially binds to alpha-2,6-linked sialic acid and mediates B-cell B-cell interactions. It plays a crucial role in activation and differentiation of the B-cell. Soluble form of CD22 (sCD22) has been found in human serum and may be useful as a tumor marker for hairy cell leukemia.

Sample Preparation

The serum, plasma, cell culture supernatants and cell lysates 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

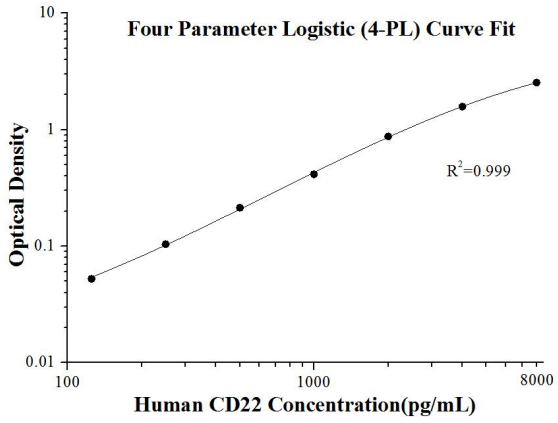
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

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.				

Example 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	0.105 0.071	0.088	-
125	0.155 0.126	0.141	0.053
250	0.201 0.183	0.192	0.104
500	0.307 0.296	0.302	0.214
1000	0.506 0.499	0.503	0.415
2000	0.953 0.969	0.961	0.873
4000	1.683 1.646	1.665	1.577
8000	2.676 2.557	2.617	2.529

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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				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	3,996.4	339.2	8.5
2	20	991.2	78.3	7.9
3	20	224.0	12.3	5.5

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	3,493.8	362.8	10.4
2	24	993.0	95.4	9.6
3	24	244.4	25.2	10.3

Recovery

The recovery of CD22 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	1:2	91	72-125
	1:4	103	87-118
Cell culture supernatants	1:2	90	77-103
	1:4	83	75-90
Cell lysates	1:4	82	71-91
	1:8	98	85-112

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Sample Values

Serum and plasma samples from healthy volunteers (human) were evaluated for CD22 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	Range (pg/mL)
Human serum (n=24)	312.7	88.7-444.0
Human plasma (n=24)	313.7	182.0-566.6

Cell lysates

Sample Type	CD22 (pg/mL)	Total protein (mg/mL)
Raji cell lysates	5,212.4	7.9
Ramos cell lysates	2,193.3	3.1
Daudi cell lysates	5,423.9	9.0

Sensitivity

The minimum detectable dose of human CD22 is 4.6 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, serum and cell culture supernatants samples were spiked with high concentrations of human CD22 in various matrices and diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. Cell lysates were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatants	Cell lysates
1:2	Average% of Expected	108	109	100
	Range (%)	98-116	89-129	-
1:4	Average% of Expected	114	97	116
	Range (%)	106-118	86-113	112-122
1:8	Average% of Expected	112	92	124
	Range (%)	108-114	83-104	110-131
1:16	Average% of Expected	112	91	104
	Range (%)	109-115	88-96	74-121

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References

1. Clark EA, et al. CD22, a B cell-specific receptor, mediates adhesion and signal transduction. J Immunol. 150(11):4715-8.
2. Nitschke L, et al. CD22 is a negative regulator of B-cell receptor signalling. Curr Biol. 7(2):133-43.
3. Carnahan J, et al. Epratuzumab, a humanized monoclonal antibody targeting CD22: characterization of in vitro properties. Clin Cancer Res. 9(10 Pt 2):3982S-90S.
4. Matsushita K, et al. Soluble CD22 as a tumor marker for hairy cell leukemia. Blood. 112(6):2272-7.

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