Proteintech

Antibodies | ELISA kits | Proteins

Human CD22 Sandwich ELISA Kit Datasheet

Catalogue Number: KE00131

Size: 5*96T

Sensitivity: 4.6 pg/mL

Range: 125-8000 pg/mL

Usage: For the quantitative detection of human CD22 concentrations in serum, plasma, cell culture supernatant and cell lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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1. 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.

2. Principle

	Capture antibody
	Target
	Detection antibody, biotinylated
	Streptavidin-HRP
	TMB
	Stop Solution

Sandwich ELISA structure (Detection antibody labeled with biotin)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with biotin also binds to the analyte. Streptavidin-HRP binds to the biotin. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	5 plates	**Unopened Kit:**
Protein standard - 16000 pg/bottle; lyophilized	10 bottles	
Detection antibody, biotinylated (100×) - 600 μL/vial*	1 vial	Store at 2-8°C for 6 months or -20°C for 12 months.
Streptavidin-horseradish peroxidase (HRP) (100×) - 600 μL/vial*	1 vial	
Sample Diluent PT 1 - 150 mL/bottle	1 bottle	**Opened Kit:**
Detection Diluent - 150 mL/bottle	1 bottle	All reagents stored at 2-8°C for 7 days.
Wash Buffer Concentrate (20×) - 150 mL/bottle	1 bottle	
Extraction Reagent - 150 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 60 mL/bottle	1 bottle	**Please use a new standard for each assay.**
Stop Solution - 60 mL/bottle	1 bottle	
Plate Cover Seals	15 pieces	

* Centrifugation immediately before use

5. Safety Notes

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

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

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6. Sample Collection and Storage

6.1 Serum: Allow blood samples to clot for 30 minutes, followed by centrifugation for 15 minutes at 1000xg. Clear serum can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.2 Plasma: Use EDTA, heparin, or citrate as an anticoagulant for plasma collection. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. The plasma can be assayed immediately or aliquoted and stored at -20℃. Avoid repeated freeze-thaw cycles.

6.3 Cell Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles.

6.4 Cell Lysate:

1) Collect cells and wash by centrifuging at 500 x g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.

2) Count cells and then discard the supernatant.

3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.

4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1 x 107 cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples.

5) Centrifuge cell lysate at 10,000 x g for 5 minutes at 4℃.

6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μL 100X Detection Antibody + 990 μL Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).
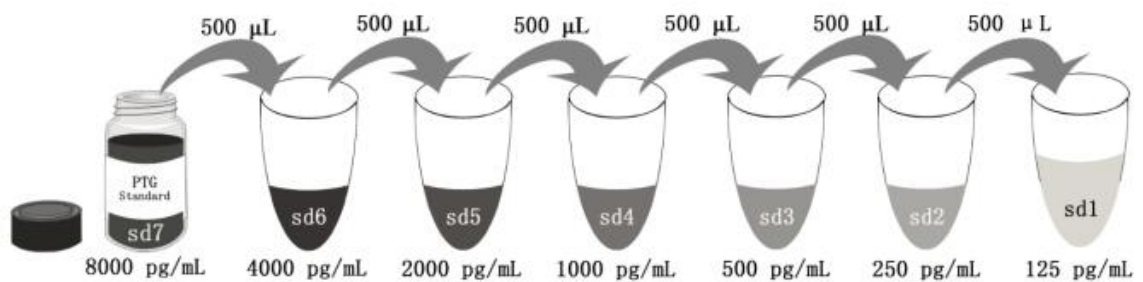
7.3 Streptavidin-HRP (1X): Dilute 100X Streptavidin-HRP 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μL 100X Streptavidin-HRP + 990 μL Detection Diluent (Centrifuge the 100X Streptavidin-HRP solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 or 1:4 is recommended for human serum and plasma; 1:2 or 1:4 is recommended for cell culture supernatant and cell lysate.

7.5 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 1 in protein standard.

	8000 pg/mL	4000 pg/mL	2000 pg/mL	1000 pg/mL	500 pg/mL	250 pg/mL	125 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"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and Streptavidin-HRP can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4℃ immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 μL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37℃.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 μL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 μL of 1X Detection Antibody solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37℃.

8.6 Repeat wash step in 8.4.

8.7 Add 100 μL of 1X Streptavidin-HRP solution (refer to Reagent Preparation7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37℃ .

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 μL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 μL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μL	120 min	4 times	Cover Wells incubate at 37℃
2	Diluent Antibody Solution	100 μL	60 min	4 times	Cover Wells incubate at 37℃
3	Diluent HRP Solution	100 μL	40 min	4 times	Cover Wells incubate at 37℃
4	TMB Substrate	100 μL	15-20 min	Do not wash	Incubate in the dark at 37℃
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.				

9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.
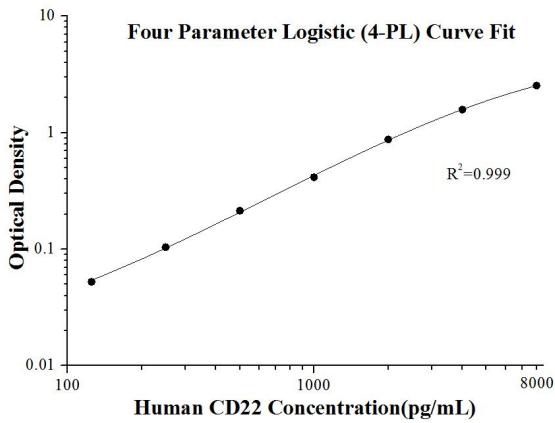
(pg/mL)	O.D	Average	Corrected
0	0.105 0.071	0.088	-
125	0.155 0.126	0.141	0.053
250	0.201 0.183	0.192	0.104
500	0.307 0.296	0.302	0.214
1000	0.506 0.499	0.503	0.415
2000	0.953 0.969	0.961	0.873
4000	1.683 1.646	1.665	1.577
8000	2.676 2.557	2.617	2.529

9.2 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

9.3 Recovery

The recovery of human CD22 spiked to three different levels 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 supernatant	1:2		90	77-103
	1:4		83	75-90
Cell lysate	1:4		82	71-91
	1:8		98	85-112

9.4 Sample values

Human serum and plasma samples from healthy volunteers (human) were evaluated for human 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 lysate

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

9.5 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.

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9.6 Linearity

To assess the linearity of the assay, human serum and cell culture supernatant 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 lysate was diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Cell culture supernatant	Cell lysate
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

10. 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.