

Speedy™ Human CD33 One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE50162

Size: 96T

Sensitivity: 11.7 pg/mL

Range: 125-8000 pg/mL; 250-16000 pg/mL

Usage: For the quantitative detection of human CD33 concentrations in serum, plasma and cell lysate.

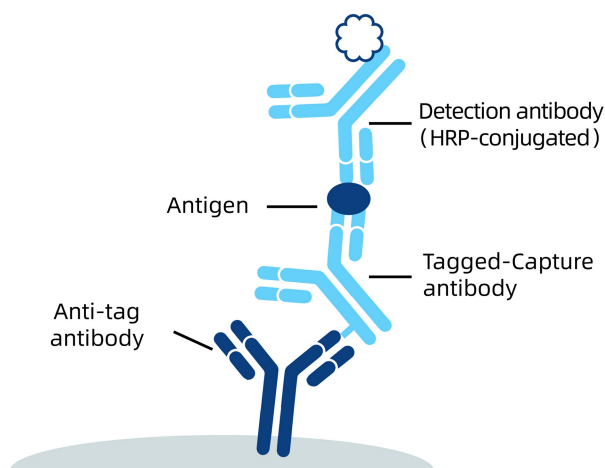
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1. Background

CD33 is a type I transmembrane receptor glycoprotein that functions as an immune inhibitory receptor. It is expressed on myeloid cells, including monocytes, macrophages, and microglia, and plays a role in immune regulation and cell adhesion. CD33 is involved in the suppression of immune responses, particularly in the context of Alzheimer's disease, where it inhibits microglial uptake of amyloid beta. Additionally, CD33 is implicated in the regulation of inflammation and immune cell activation.

2. Principle



An anti-tag antibody is pre-coated onto the bottom of wells. After adding antigen or samples, Tagged-Capture antibody and HRP-conjugated detection antibody, a sandwich complex is formed in the solution. TMB acts as a HRP substrate, and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns the 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. Proteintech data analysis website, <https://www.ptgcn.com/products/elisa-kits/>.
- 3.8 Microplate thermostatic shaker.

4. Kit Components and Storage

Microplate - 96 well microplate precoated an anti-tag antibody (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	
Capture antibody (100×) - 60 µL/vial*	1 vial	
Detection antibody, HRP-conjugated (100×) - 60 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 30 mL/bottle. For human serum and plasma samples.	1 bottle	
Sample Diluent PT 3-ef - 30 mL/bottle. For cell lysate samples.	1 bottle	
Detection Diluent - 15 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 15 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 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

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°C. 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°C. Avoid repeated freeze-thaw cycles.

6.3 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 1×10^7 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°C.
- 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 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 μ L 100X capture antibody + 50 μ L 100X Detection Antibody, HRP-conjugated + 4,900 μ L Detection Diluent. Mix gently but thoroughly.

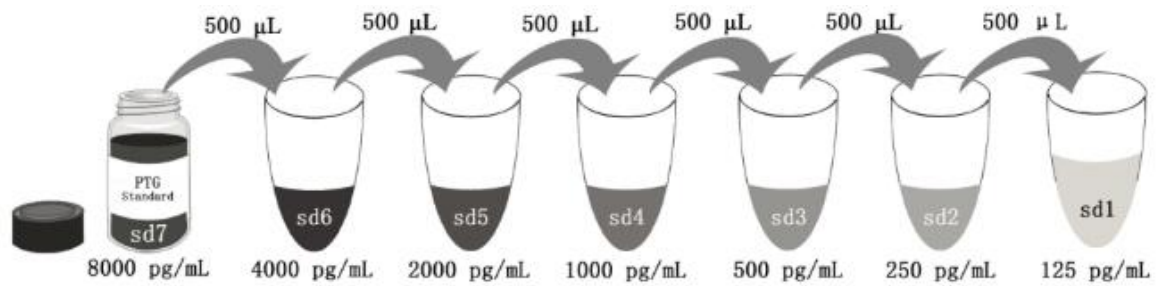
7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent. The minimum required dilution factor was 1:2; consequently, no more than 25 μ L of undiluted sample was added per well.

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; 1:16 or 1:32 is recommended for cell lysate.

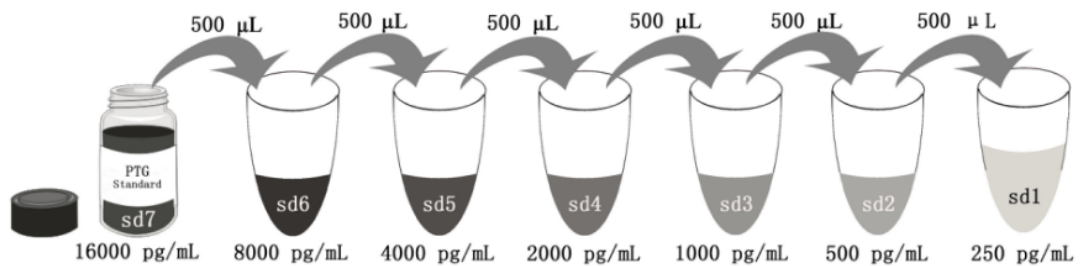
7.4 Standard Serial Dilution:

For human serum and plasma samples, add 2 mL Sample Diluent PT 4B1 in protein standard.



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 4B1	2000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

For cell lysate samples, add 1 mL Sample Diluent PT 3-ef in protein standard.



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 3-ef	1000 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, HRP-conjugated antibody 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 Preset the layout of the microplate, including control group, standard group and sample group, 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°C immediately. Microplate strips should be used in one week.

8.2 Add 50 µL standard or sample to appropriate wells. To avoid high background always add samples or standards to the well before the addition of antibody cocktail.

8.3 Add 50 µL 1× Antibody Cocktail solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 1 hour (incubate at 37°C for 2 hours is recommended if thermostatic shaker is not available) .

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 1× 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 TMB substrate solution to each well, protected from light. Incubate at 37°C on a microplate thermostatic shaker set at 400 rpm for 15 to 20 minutes. (Substrate Solution should remain colorless until added to the plate.)

8.6 Add 100 µL Stop Solution to each well in the same order as addition of the TMB substrate. Note: Avoid skin and eye contact with the Stop solution.

8.7 Read results immediately on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.8 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, four-parameter logistic curve-fit (4-PL) analysis is recommended. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

Add 50 μ L standard or sample to appropriate wells



Add 50 μ L antibody cocktail solution (1x) to each well



Incubate at 37 $^{\circ}$ C on a microplate thermostatic shaker set at 400 rpm for 1 hour



Aspirate and wash each well four times with 400 μ L 1 x Wash Buffer



Add 100 μ L TMB substrate solution to each well



Incubate at 37 $^{\circ}$ C on a microplate thermostatic shaker set at 400 rpm for 15minutes

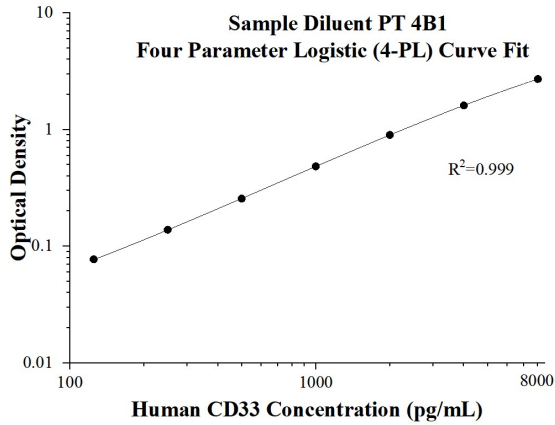


Add 100 μ L Stop Solution to each well and Read OD immediately

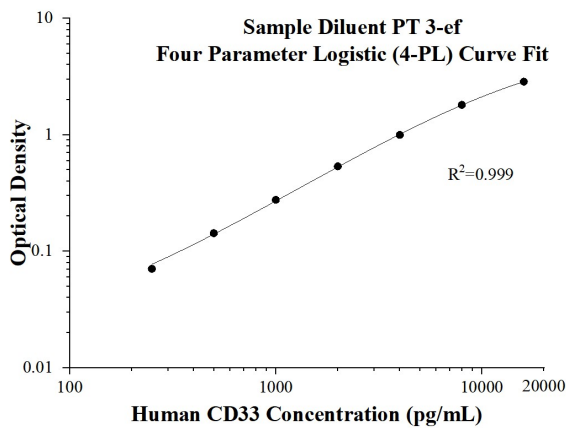
9. Validation Data

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	0.0642 0.064	0.0641	-
125	0.1304 0.1522	0.1413	0.0772
250	0.1991 0.2058	0.20245	0.13835
500	0.3113 0.3285	0.3199	0.2558
1000	0.55 0.5463	0.54815	0.48405
2000	0.9589 0.9687	0.9638	0.8997
4000	1.6765 1.6762	1.67635	1.61225
8000	2.8456 2.7057	2.77565	2.71155



(pg/mL)	O.D	Average	Corrected
0	0.0341 0.0344	0.03425	0
250	0.1055 0.1044	0.10495	0.0707
500	0.1781 0.1762	0.17715	0.1429
1000	0.3142 0.3062	0.3102	0.27595
2000	0.5671 0.572	0.56955	0.5353
4000	1.0392 1.0237	1.03145	0.9972
8000	1.8312 1.8528	1.842	1.80775
16000	2.8865 2.9021	2.8943	2.86005

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision					Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%	Sample	n	Mean (pg/mL)	SD	CV%
1	8	4.86	0.18	3.70	1	16	4.78	0.16	3.35
2	8	1.03	0.08	7.77	2	16	1.03	0.09	8.74
3	8	0.51	0.02	3.92	3	16	0.51	0.02	3.92

9.3 Recovery

The recovery of human CD33 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:8	119	113-122
	1:16	120	114-127
Cell lysate	1:64	92	91-94
	1:128	97	94-98

9.4 Sample values

Human plasma - Human plasma samples were evaluated for the presence of human CD33 in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Human plasma (n=16)	2521.1	1213.1-6538.5

Cell lysate

	Human CD33 (ng/mL)	Total protein (mg/mL)
THP-1 cell lysate	40.7	2.3

9.5 Sensitivity

The minimum detectable dose of human CD33 is 11.7 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay.

(The cell lysate was initially diluted 1:8.)

		Human plasma (Sample diluent PT 4B1)	Cell lysate (Sample diluent PT 3-ef)
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	117	112
	Range (%)	116-118	107-116
1:8	Average% of Expected	129	108
	Range (%)	126-132	103-113
1:16	Average% of Expected	112	84
	Range (%)	108-117	79-90

9.7 Specificity

This kit specifically recognizes native and recombinant human CD33.

10. References

1. Jiang, Teng et al. Molecular neurobiology vol. 49,1 (2014): 529-35.
2. Estus, Steven et al. Acta neuropathologica vol. 138,2 (2019): 187-199.
3. Wißfeld, Jannis et al. Glia vol. 69,6 (2021): 1393-1412.