Speedy™ Mouse CD31 One-Step ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: SE60054

Size: 96T

Sensitivity: 4.2 pg/mL

Range: 78.1-5000 pg/mL

Usage: For the quantitative detection of mouse CD31 concentrations in serum, plasma, cell lysate and tissue lysate.

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

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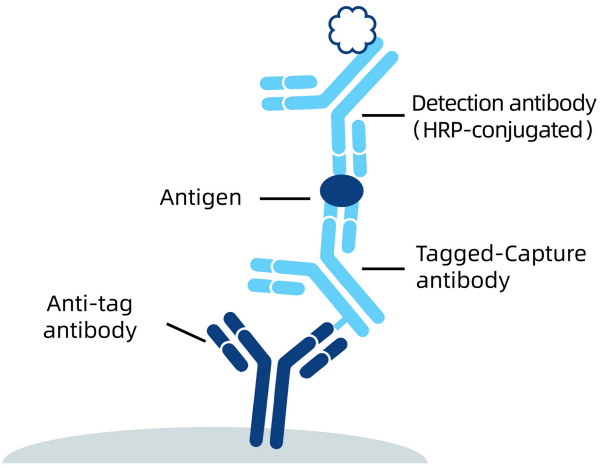
1. Background

CD31, also known as platelet endothelial cell adhesion molecule1 (PECAM-1), is a 130 kDa platelet-endothelial cell (EC) adhesion molecule initially identified from ECs and platelets and later from blood leucocytes. CD31 was initially classified as a cell adhesion molecule, later studies suggested that CD31 triggers downstream inhibitory signaling upon trans-homophilic CD31 engagement during cell-cell interaction. CD31 signaling participates in regulating leucocyte detachment, T-cell activation, platelet activation, and angiogenesis, all of which are critical to the pathogenesis of atherosclerosis and abdominal aortic aneurysms (AAAs).

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.

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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 - 10000 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 1 - 30 mL/bottle. For mouse serum and plasma.	1 bottle	
Sample Diluent PT 4B1 - 30 mL/bottle. For cell lysate and tissue lysate.	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

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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°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 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°C.

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

6.4 Tissue Lysate:

1) Rinse tissue with PBS, cut into 1-2 mm pieces.

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

3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.

4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.

5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.

6) Measure the concentration of total protein in tissue homogenates using BCA assay.

7) Avoid protein degradation by performing all the above procedures on ice where possible.

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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.

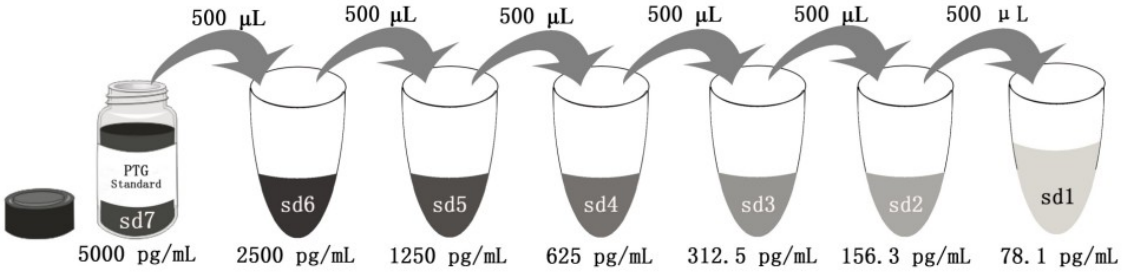
7.2 Antibody Cocktail (1X): Dilute 100X capture antibody and 100X HRP-conjugated detection antibody using Detection Diluent prior to assay. Suggested 1:100 dilution: 50 µL 100X capture antibody + 50 µL 100X Detection Antibody, HRP-conjugated + 4,900 µL Detection Diluent. Mix gently but thoroughly.

7.3 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: 1:8 or 1:16 is recommended for mouse serum and plasma; 1:200 to 1:1,600 is recommended for cell lysate and tissue lysate.

7.4 Standard Serial Dilution:

For mouse serum and plasma, add 2mL Sample Diluent PT 1 in protein standard. For cell lysate and tissue lysate, add 2mL 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 1 or PT 4B1	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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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 recommend. If the samples have been diluted, the fitting result must be multiplied by the dilution factor used.

Procedure summary

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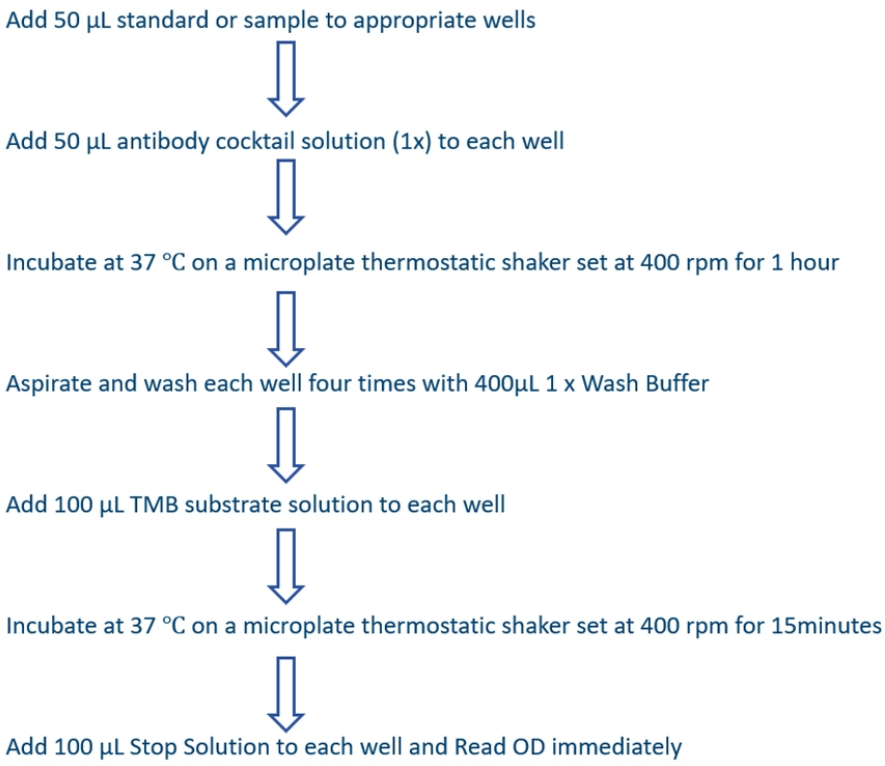

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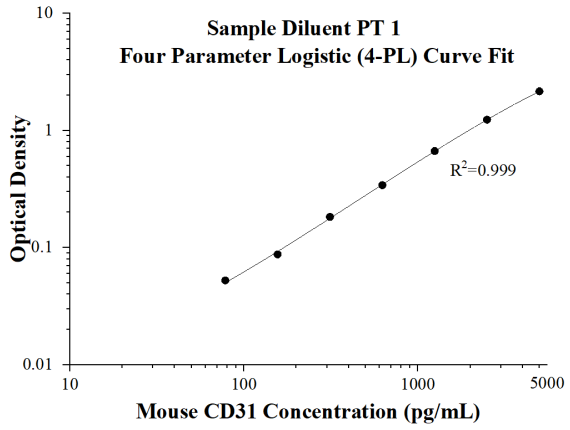
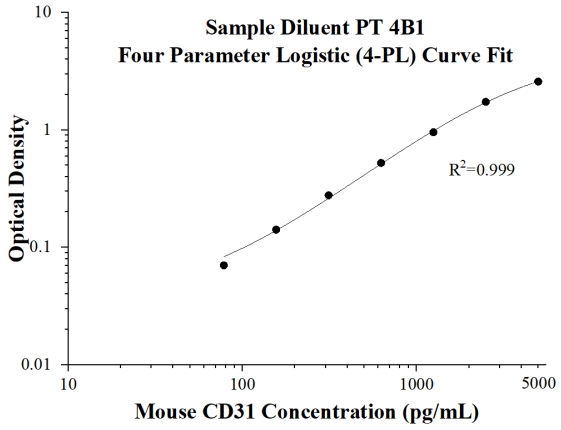
9. Validation Data

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	0.0233 0.0228	0.02305	-
78.1	0.0754 0.0758	0.0756	0.05255
156.3	0.1111 0.1102	0.11065	0.0876
312.5	0.2034 0.2084	0.2059	0.18285
625	0.3626 0.3666	0.3646	0.34155
1250	0.6743 0.7077	0.691	0.66795
2500	1.301 1.219	1.26	1.23695
5000	2.2133 2.1539	2.1836	2.16055



(pg/mL)	O.D	Average	Corrected
0	0.036 0.0342	0.0351	-
78.1	0.1062 0.1048	0.1055	0.0704
156.3	0.1804 0.1727	0.17655	0.14145
312.5	0.3249 0.3012	0.31305	0.27795
625	0.5671 0.5503	0.5587	0.5236
1250	1.0092 0.9742	0.9917	0.9566
2500	1.7914 1.7492	1.7703	1.7352
5000	2.6304 2.6153	2.62285	2.58775

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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				
Sample	n	Mean (pg/mL)	SD	CV%
1	8	2,610.8	67.5	2.6
2	8	672.2	17.2	2.6
3	8	340.1	9.0	2.6

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	16	2,576.4	75.6	2.9
2	16	669.5	18.8	2.8
3	16	341.0	11.6	3.4

9.3 Recovery

The recovery of mouse CD31 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Mouse serum	1:16	103	100-109
Lysate	1:1,600	112	99-122

9.4 Sample values

Mouse serum - mouse serum samples were evaluated for the presence of mouse CD31 in this assay.

Sample Type	Mean (**ng/mL**)	Range (**ng/mL**)
Mouse serum (n=16)	11.3	8.5-18.8

Cell/Tissue lysate

	Mouse CD31 (**ng/mL**)	Total protein (**mg/mL**)
bEnd.3 cell lysate	337.0	1.2
Mouse lung tissue lysate	1,130.0	5.0

9.5 Sensitivity

The minimum detectable dose of mouse CD31 is 4.2 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, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(The mouse serum was initially diluted 1:4. The lysate was initially diluted 1:400.)

		Mouse serum (Sample Diluent PT 1)	Lysate (Sample Diluent PT 4B1)
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	91	98
	Range (%)	88-94	98-99
1:8	Average% of Expected	81	101
	Range (%)	76-85	100-102
1:16	Average% of Expected	78	93
	Range (%)	74-82	84-102

9.7 Specificity

This assay recognizes natural and recombinant mouse CD31.

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

CD31

10. References

1. Van Mourik JA. et al. (1985) J Biol Chem. 260:11300–11306.
2. Stockinger H. et al. (1990) J Immunol. 145:3889–3897.
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