Human Endoglin/CD105 Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00335

Size: 5*96T

Sensitivity: 0.01 ng/mL

Range: 0.156-10 ng/mL

Usage: For the quantitative detection of human Endoglin/CD105 concentrations in serum, plasma, cell culture supernatant, 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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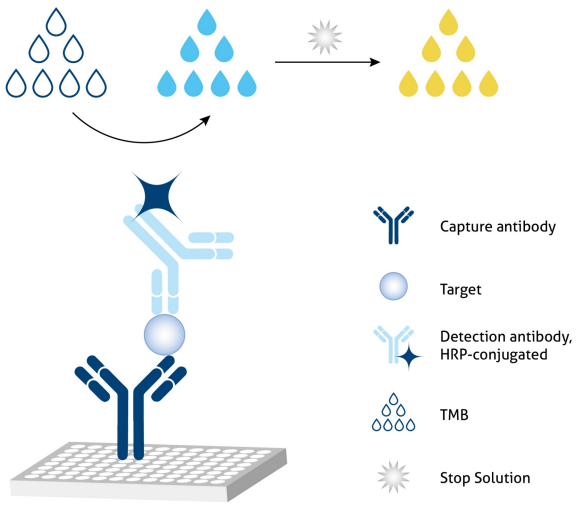
1. Background

Endoglin (ENG, CD105) is a homodimeric cell membrane glycoprotein of 180 kDa, composed of disulphide-linked subunits of 90-95 kDa. Endoglin is a proliferation-associated and hypoxia-inducible protein mainly expressed on vascular endothelial cells. It acts as an accessory receptor for transforming growth factor beta (TFG-β) and is involved in vascular development and remodelling. The important role of Endoglin in angiogenesis and in tumor progression makes it an ideal target for antiangiogenic therapy and a good marker for tumor prognosis. The extracellular domain of membrane-bound Endoglin can be proteolytically cleaved, releasing a soluble form of Endoglin (sCD105). Increased levels of sCD105 are linked to the pathogenesis of severe vascular disease, and also correlate with poor prognosis in patients suffering from various types of cancer.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with HRP)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. 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.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	5 plates	**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 - 20 ng/bottle; lyophilized	10 bottles	
Detection antibody, HRP-conjugated (100×) - 600 µL/vial*	1 vial	
Sample Diluent PT 4B1 - 150 mL/bottle.	1 bottle	
Detection Diluent - 150 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 150 mL/bottle	1 bottle	
Extraction Reagent - 150 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 60 mL/bottle	1 bottle	
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°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 Culture Supernatant: Remove particulates by centrifugation for 5 minutes at 500xg and assay immediately or aliquot and store samples at ≤ -20°C. 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°C.

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

6.5 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.

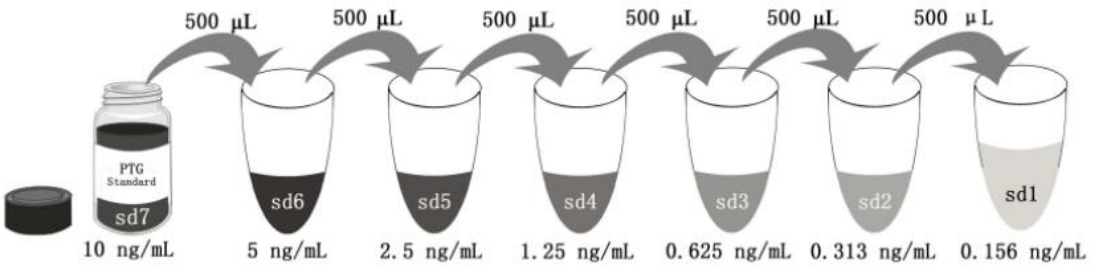
7.2 Detection Antibody, HRP-conjugated(1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 µL 100X Detection Antibody, HRP-conjugated + 990 µL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated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

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: 1:4 is recommended for serum and plasma; 1:2 is recommended for cell culture supernatant .

7.4 Standard Serial Dilution:

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"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated 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°C 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°C.

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, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.6 Repeat wash step in 8.4.

8.7 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.

8.8 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.

8.9 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).

8.10 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°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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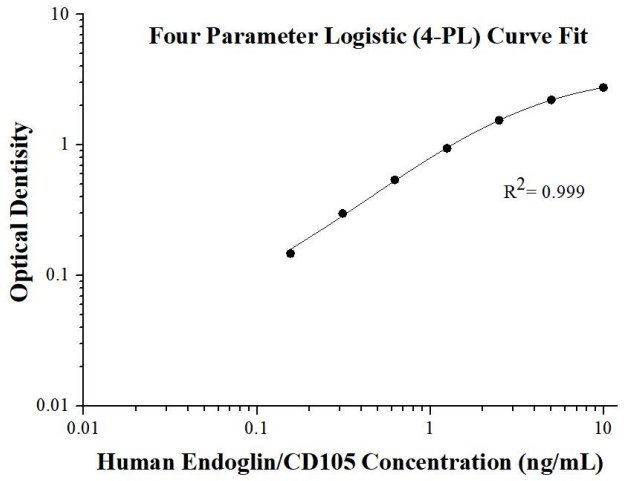
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.0046 0.0051	0.0049	-
0.156	0.1500 0.1553	0.1527	0.1478
0.313	0.3078 0.3003	0.3041	0.2992
0.625	0.5544 0.5371	0.5458	0.5409
1.25	0.9602 0.9371	0.9487	0.9438
2.5	1.5977 1.5074	1.5526	1.5477
5	2.2750 2.1751	2.2251	2.2202
10	2.7738 2.753	2.7634	2.7585

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 (ng/mL)	SD	CV%
1	20	5.62	0.33	5.94
2	20	1.13	0.04	3.19
3	20	0.30	0.01	3.63

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	5.52	0.28	5.09
2	24	1.12	0.04	3.19
3	24	0.31	0.01	2.88

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9.3 Recovery

The recovery of human Endoglin/CD105 spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type		Average % of Expected	Range (%)
Human plasma	1:16	100	87-114
	1:32	100	87-112
Cell culture supernatant	1:2	102	95-113
	1:4	100	90-106

9.4 Sample values

Human plasma - Human plasma samples were evaluated for the presence of human Endoglin/CD105 in this assay.

Sample Type	Mean (ng/mL)	Rang (ng/mL)
Human plasma (n=16)	7.05	4.06-10.50

Cell culture supernatant - Human peripheral blood mononuclear cells (PBMC) ($1x10^6$ cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin sulfate and stimulated with 10 µg/mL PHA. Aliquots of the culture supernatants were removed on 5 days and assayed for levels of human Endoglin/CD105.

Condition	Day 5 (**pg/mL**)
Unstimulated	40.57
Stimulated	113.77

9.5 Sensitivity

The minimum detectable dose of human Endoglin/CD105 is 0.01 ng/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 plasma and cell culture supernatant were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

(Human plasma samples were initially diluted 1:2.)

		Human plasma	Cell culture supernatant
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	99	99
	Range (%)	87-105	97-102
1:8	Average% of Expected	103	92
	Range (%)	90-111	73-110
1:16	Average% of Expected	102	98
	Range (%)	96-109	98

9.7 Specificity

This assay recognizes natural and recombinant human Endoglin/CD105.

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

Recombinant human:

BMP-2

BMP-4

TNG-β1

TNG-β2

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10. References

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