# Human Endoglin/CD105 Sandwich ELISA Kit Datasheet

For the quantitative detection of human Endoglin/CD105in serum, plasma and cell culture supernatants.

## General Information

| | |
|---|---|
| **Catalogue Number** | KE00199 |
| **Product Name** | Human Endoglin/CD105 Sandwich ELISA Kit |
| **Species cross-reactivity** | Human |
| **Range (calibration Range)** | 31.25-2000 pg/mL |
| **Tested applications** | Quantification ELISA |

## Database Links

| | |
|---|---|
| **Entrez Gene** | 2022 |
| **SwissProt** | P17813 |

## 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** - 2000 pg/bottle; lyophilized* | 2 bottles | |
| **Detection antibody, biotinylated (100X)** - 120 µL/vial | 1 vial | |
| **Streptavidin-horseradish peroxidase (HRP) (100X)** - 120 µL/vial | 1 vial | |
| **Sample Diluent PT 4-ef** - 30 mL/bottle. | 1 bottle | |
| **Detection Diluent** - 30 mL/bottle | 1 bottle | |
| **Wash Buffer Concentrate (20X)** - 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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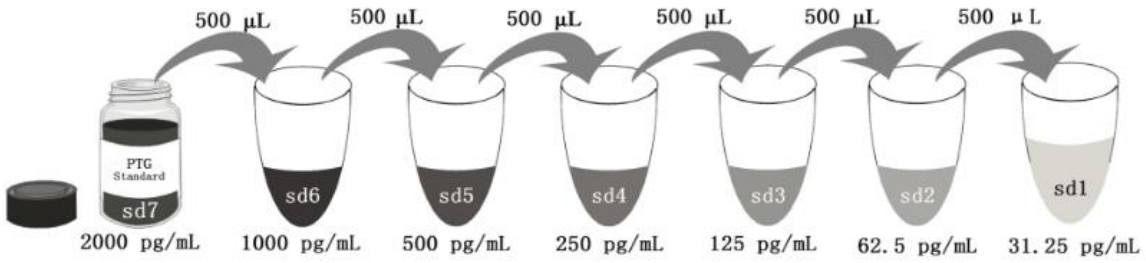
**NB: Do not use the kit after the expiration date.**
Sample Diluent PT 4-ef is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 4-ef in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.




| 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 4-ef | **1000 µL** | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL | 500 µL |
| | "sd7" | "sd6" | "sd5" | "sd4" | "sd3" | "sd2" | "sd1" |

## Product Description

KE00199 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The Endoglin/CD105 ELISA kit is to be used to detect and quantify protein levels of endogenous Endoglin/CD105. The assay recognizes human Endoglin/CD105. An antibody specific for Endoglin/CD105 has been pre-coated onto the microwells. The Endoglin/CD105 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for Endoglin/CD105 is added to detect the captured Endoglin/CD105 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

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.

## Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. 1:8 or 1:16 dilution is



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recommended for serum or plasma. 1:2 dilution is recommended for cell culture supernatants.

## Safety Notes

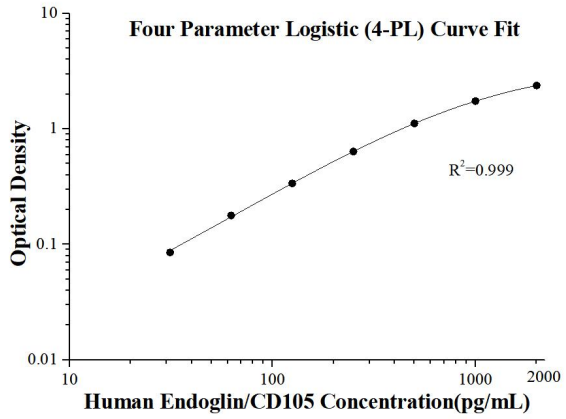
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

## 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 | 0.096<br>0.094 | 0.095 | - |
| 31.25 | 0.183<br>0.177 | 0.180 | 0.085 |
| 62.5 | 0.277<br>0.269 | 0.273 | 0.178 |
| 125 | 0.433<br>0.432 | 0.433 | 0.338 |
| 250 | 0.736<br>0.727 | 0.732 | 0.637 |
| 500 | 1.216<br>1.205 | 1.211 | 1.116 |
| 1000 | 1.886<br>1.792 | 1.839 | 1.744 |
| 2000 | 2.053<br>2.448 | 2.476 | 2.381 |



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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 | 999.7 | 33.7 | 3.4 |
| 2 | 20 | 222.4 | 3.8 | 1.7 |
| 3 | 20 | 51.3 | 2.1 | 4.1 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (pg/mL) | SD | CV% |
| 1 | 24 | 1,011.0 | 24.3 | 2.4 |
| 2 | 24 | 227.9 | 4.9 | 2.1 |
| 3 | 24 | 51.0 | 3.7 | 7.2 |

## Recovery

The recovery of Endoglin/CD105 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 | 1:30 | 83 | 80-88 |
| | 1:60 | 85 | 80-94 |
| Cell culture supernatants | 1:2 | 81 | 77-84 |
| | 1:4 | 85 | 78-94 |

## Sample Values

Human serum samples from healthy volunteers were evaluated for Endoglin/CD105 in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean (ng/mL) | Range (ng/mL) |
|---|---|---|
| Human serum (n=16 ) | 3.43 | 2.84-4.33 |

**cell culture supernatants:**

Human peripheral blood mononuclear cells (1 x $10^6$ cells/mL) were cultured in RPMI supplemented with 8% fetal bovine serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin and 100 μ g/mL streptomycin sulfate. Cells were stimulated with 10 ug/mL PHA. Aliquots of the cell culture supernatants were removed and assayed for levels of human Endoglin/CD105.

| Condition | Day 1 (pg/mL) | Day 5 (pg/mL) |
|---|---|---|
| Unstimulated | ND | ND |
| Stimulated | ND | 69.13 |

ND=Non-detectable



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## Sensitivity

The minimum detectable dose of human Endoglin/CD105 is 2.5 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 samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay, cell culture supernatants samples were spiked with high concentrations of human Endoglin/CD105 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The serum samples were initially diluted 1:8 )

| | | Human serum | Cell culture supernatants |
|---|---|---|---|
| 1:2 | Average% of Expected | 100 | 94 |
| | Range (%) | - | 85-100 |
| 1:4 | Average% of Expected | 106 | 95 |
| | Range (%) | 104-108 | 89-99 |
| 1:8 | Average% of Expected | 105 | 99 |
| | Range (%) | 101-111 | 95-102 |
| 1:16 | Average% of Expected | 100 | 97 |
| | Range (%) | 93-105 | 91-102 |

## References

1. Cheifetz S, Bellón T, Calés C, et al. Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells[J]. Journal of Biological Chemistry, 1992, 267(27): 19027-19030.
2. Fonsatti E, Altomonte M, Nicotra M R, et al. Endoglin (CD105): a powerful therapeutic target on tumor-associated angiogenetic blood vessels[J]. Oncogene, 2003, 22(42): 6557-6563.
3. Nassiri F, Cusimano M D, Scheithauer B W, et al. Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy[J]. Anticancer research, 2011, 31(6): 2283-2290.
4. Duff S E, Li C, Garland J M, et al. CD105 is important for angiogenesis: evidence and potential applications[J]. The FASEB Journal, 2003, 17(9): 984-992.
5. Pappa C A, Alexandrakis M G, Boula A, et al. Emerging roles of endoglin/CD105 and angiogenic cytokines for disease development and progression in multiple myeloma patients[J]. Hematological oncology, 2013, 31(4): 201-205.



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