HUMAN SOLUBLE ENDOGLIN / CD105 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ENDOGLIN/CD105 CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE ENDOGLIN/CD105 ELISA
Catalog No.	SK00697-01
Lot No.	
Formulation	96 T
Standard Range	125-8000 pg/mL
Sensitivity	62 pg/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human Endoglin
Dilution Factor	10 **(Optimal dilutions should be determined by each laboratory for each application)**
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 10%
Storage	2 - 8° C

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human Soluble Endoglin/CD105 immunoassay is a solid phase ELISA designed to measure human Endoglin in cell culture supernates, serum, and plasma. It contains recombinant human Endoglin and antibodies raised against this protein. It has been shown to accurately quantify recombinant human Endoglin. Results obtained with naturally occurring Endoglin samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human Endoglin.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Endoglin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Endoglin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for Endoglin is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of Endoglin bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Endoglin Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against Endoglin.	**697-01-01**	**1 plate**
Endoglin Standard – 8000 pg/vial of recombinant human Endoglin in a buffered protein base with preservative; lyophilized.	**697-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05mL/vial, 10-fold concentrate of biotinylated antibody against Endoglin with preservative; lyophilized.	**697-01-03**	**1 vial**
Positive Control - one vial of recombinant human Endoglin; lyophilized.	**697-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Standard and Detection Antibody: Reconstituted standard (stock) and Detection Antibody concentrated solution could be

stored for up to one month at -20° C or -70° C. Streptavidin-HRP Conjugate 200-fold concentrated solution **(protect from light)** and other components may be stored at 2 – 8° C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum and plasma samples may require a 10-fold dilution. A suggested 10-fold dilution is 25 µL sample + 225 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

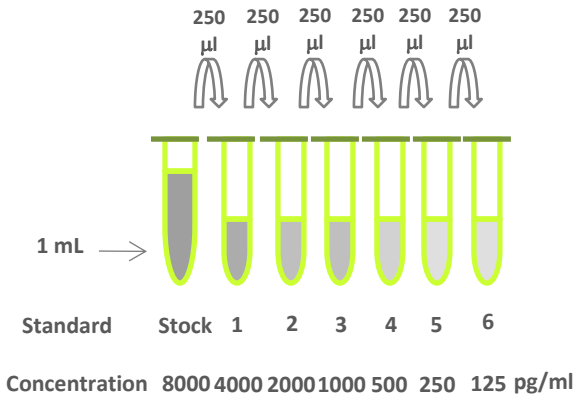
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Endoglin Standard - Refer to vial label for reconstitution volume. Reconstitute the Endoglin standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 µl	8000 pg/ml
# 1	250 µl of stock	250 µl	4000 pg/ml
# 2	250 µl of 1	250 µl	2000 pg/ml
# 3	250 µl of 2	250 µl	1000 pg/ml
# 4	250 µl of 3	250 µl	500 pg/ml
# 5	250 µl of 4	250 µl	250 pg/ml
# 6	250 µl of 5	250 µl	125 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

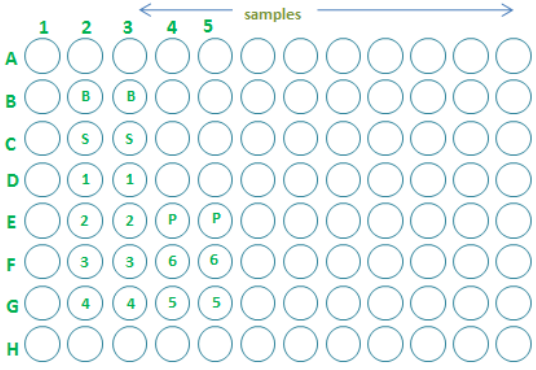
Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note**: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note**: Positive Control could be reused within a few days if stored at -20°C or -70° C.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard solutions in reverse order of serial dilution (from F4, F5 to G4, G5 and G2, G3 to C2, C3), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 3-6 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer

software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Endoglin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 8000 pg/mL may result in inaccurate, low human Endoglin/CD105 levels. Such samples require further external predilution according to expected human Endoglin/CD105 values with Dilution Buffer in order to precisely quantify the actual human Endoglin/CD105 level.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Endoglin.

SENSITIVITY

The minimum detectable dose (MDD) of Endoglin was 62 pg/mL.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Endoglin/CD105 (pg/mL)	Average OD450 (Corrected)*
Blank	0 (0.145)
125	0.079
250	0.160
500	0.355
1000	0.680
2000	1.298
4000	1.866
8000	2.532

- Lot No.:
- Positive Control:

LINEARITY

To assess the linearity of the assayed, pooled research human **serum** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
10x	2762.126	27621.26	100
20x	1474.510	29490.20	107

To assess the linearity of the assayed, pooled research human **EDTA plasma** samples were diluted with Dilution Buffer and assayed.

DILUTION FACTOR	ASSAYED (PG/ML)	FINAL (PG/ML)	RECOVERY (%)
10x	1991.906	19919.06	100
20x	1053.467	21069.34	106

SPECIFICITY

This assay recognizes both natural and recombinant human Endoglin. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh Endoglin control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY
Human Endoglin	100%
Mouse Endoglin/Fc Chima	0
Human Activin A	0
Human TGF-beta 1	0
Human Lipocalin 2	0
Human Soluble CD36	0
Human Soluble CD320	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 3-6 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 15 min.