

# HUMAN SOLUBLE CD305 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION  
OF HUMAN SOLUBLE CD305  
CONCENTRATIONS IN SERUM AND  
PLASMA



THIS PROTOCOL OR DATA IS PROVIDED  
FOR DEMONSTRATION ONLY.  
ALWAYS REFER TO LOT SPECIFIC PROTOCOL  
PROVIDED WITH EACH KIT FOR  
INSTRUCTIONS. PROTOCOL MUST BE  
READ BEFORE USING THIS PRODUCT.

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

## PRODUCT INFORMATION:

**THIS KIT IS FOR ONE TIME USE ONLY.**

ELISA NAME	HUMAN SOLUBLE CD305 ELISA
Catalog No.	SK00410-01
Lot No.	
Formulation	96 T
Standard range	62.5 – 4000 pg/mL
Sensitivity	30 pg/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma
Dilution Factor	<b>Optimal dilutions should be determined by each laboratory for each application</b>
Specificity	Human sCD305
Calibration	Human sCD305 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C for 1 month. See page 3 for detail
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

## ORDER CONTACT:

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**DESCRIPTION**

This Human Soluble CD305 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Soluble CD305 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human Soluble CD305 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Soluble CD305 samples.

**ASSAY OVERVIEW**

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human Soluble CD305. The capture antibody can bind to the human Soluble CD305 in the standard and samples. After washing the plate of any unbound substances, a rabbit antibody against human Soluble CD305 is added to the wells. After another washing of the plate, Anti Rabbit IGG-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human Soluble CD305 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

**PROCEDURAL LIMITATIONS**

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

\_This ELISA kit should not be used beyond the expiration date on the kit label.

\_Do not mix 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.

\_Each laboratory must determine the optimal dilution factors for the samples being assayed.

\_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

\_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

**COMPONENTS PROVIDED**

DESCRIPTION	CODE	QUANTITY
<b>Soluble CD305 Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Soluble CD305.	<b>410-01-01</b>	<b>1 plate</b>
<b>Soluble CD305 Standard</b> – refer to lot of recombinant human Soluble CD305 in a buffered protein base with preservative; lyophilized.	<b>410-01-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – refer to lot, 10-fold concentrate of rabbit antibody against Soluble CD305 with preservative; lyophilized.	<b>410-01-03</b>	<b>1 vial</b>
<b>Positive Control Concentrate</b> - one vial recombinant human Soluble CD305 with preservative; lyophilized.	<b>410-01-04</b>	<b>1 vial</b>
<b>Anti Rabbit IGG-HRP Conjugate</b> - 120 µL/vial, 100-fold concentrated solution of Anti Rabbit IGG-HRP conjugate.	<b>ARIGHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> - 30 mL of buffered protein based solution with preservative.	<b>DB06</b>	<b>1 bottle</b>
<b>Antibody Diluent Solution</b> – 12 mL of buffered protein based solution with preservative.	<b>DB16</b>	<b>1 bottle</b>
<b>HRP Diluent Solution</b> – 12 mL of buffered protein based solution with preservative.	<b>DB08A</b>	<b>1 bottle</b>
<b>Wash Buffer</b> - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> -11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> - 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>

Plate Sealer	EAPS	1
Plastic Pouch	P01	1

**STORAGE**

**Unopened Kit:** Store at 2 – 8° C for up to 1 month. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer, Antibody Diluent Solution and HRP Diluent Solution should be stored at -20° C. Anti Rabbit IGG-HRP conjugate and TMB Substrate Solution should be stored only at 2 -8 °C. Do not use kit past expiration date.

**ADDITIONAL MATERIALS REQUIRED**

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

**PRECAUTION**

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

**SAMPLE COLLECTION AND STORAGE**

**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**

Plasma and serum samples DOES NOT need to be diluted. **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.

**Soluble CD305 Standard** - Reconstitute the soluble CD305 standard with refer to lot of Dilution Buffer. Pipette 250 µL of Dilution Buffer into tubes #2 to #7. Use the high standard of **4000 pg/mL** to produce a dilution series. Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Refer to lot	
# 1	Refer to lot	Refer to lot	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
# 7	250 µl of 6	250 µl	62.5 pg/ml

**Positive Control** - Reconstitute the Positive Control Concentrate with refer to lot of Dilution Buffer to prepare working solution for Positive Control test.

**Detection Antibody Concentrate** - Reconstitute the Detection Antibody Concentrate with 1refer to lot of **Antibody Diluent Solution (DB16)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB16)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

**Anti Rabbit IGG-HRP Conjugate** - Transfer 120 µL of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution (DB08A)** to prepare working solution. **(Protect from light.)**

**ELISA PROTOCOL**

**Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL of **Standard dilutions, samples, or positive control** per well. Cover with plate sealer. Incubate for 2 hours on plate shaker at room temperature.
4. 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 auto-washer. 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.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on plate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Anti Rabbit IGG-HRP Conjugate working solution** to each well. Incubate for 60 minutes on plate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for refer to lot on a plate shaker at room temperature. **Protect from light.**
10. 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.
11. Determine the optical density of each well 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 Galectin-3 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.

**TYPICAL STANDARD CURVE**

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

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (refer to lot)
62.5	0.029
125	0.062
250	0.119
500	0.229
1000	0.522
2000	1.109
4000	1.725

**SPECIFICITY**

PROTEINS	CROSSREACTIVITY (%)
Human sCD305	100
Human sCD36	0
Human sCD306	0
Human sCD209	0

**SUMMARY OF ASSAY PROCEDURE**

<b>PREPARE REAGENTS, SAMPLES AND STANDARDS</b>
↓
Add 100 µL of standard dilutions, samples, or positive control to each well. Incubate for 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Detection Antibody working solution to each well. Incubate for 2 hours on plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Anti Rabbit IGG-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. <b>Protect from light.</b>
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. <b>Protect from light.</b>
↓
Add 100 µL Stop Solution to each well. Read at 450nm.