HUMAN SOLUBLE IL1RL1/IL1R4/ST2 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN IL1RL1 CONCENTRATIONS IN CELL CULTURE SUPERNATES, PLASMA AND SERUM

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS 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 IL1RL1/ST2 ELISA KIT**
Catalog No.	SK00120-01
Lot No.	
Formulation	96 T
Standard Range	62.5 - 4000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Sample Dilution	**2 -4 (Optimal dilutions should be determined by each laboratory for each application)**
Sample Type	Serum, Plasma, Cell Culture Supernates
Specificity	Human Soluble IL1RL1 only
Calibration	Human Soluble IL1RL1 Isoform B (HEK293)
Intra-assay Precision	4 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8° C for 1 month. more information check page 2
This kit contains sufficient materials to run approximately 35 samples duplicated provided that assay is run according to protocol.	

ORDER CONTACT:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.net
www.AvisceraBioscience.com

DESCRIPTION

This Human Soluble IL1RL1/IL1R4/ST2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human soluble IL1RL1 from cell culture supernates, serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant the glycosylated human soluble IL1RL1 /ST2 from HEK293 cells and two monoclonal antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural soluble IL1RL1 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human soluble IL1RL1. The capture antibody can bind to the human soluble IL1RL1 in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human soluble IL1RL1 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human soluble IL1RL1 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURE 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.
_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
Soluble IL1RL1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against human soluble IL1RL1.	**120-01-01**	**1 plate**
Soluble IL1RL1 Standard – 16 ng/vial of recombinant human **Soluble IL1RL1** in a buffered protein base with preservative; lyophilized.	**120-01-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated purified monoclonal antibody against human Soluble IL1RL1 with preservative; lyophilized.	**120-01-03**	**1 vial**
Streptavidin-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB01**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB12**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11 mL of 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 1 month. For longer storage up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C or -70° C. Streptavidin-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 (350 – 400 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

Cell Culture Supernates – Centrifuge and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Serum – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Optional: Use Aprotinin (enzyme inhibitor) (Aviscera Order Code: 00700-01-25, 25 TIU) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

SAMPLE PREPARATION

Human serum or plasma samples may need 2-4 fold dilution.
Optimal dilutions should be determined by each laboratory for each application with a pretest.
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 Il1RL1 Standard - Reconstitute the Soluble IL1RL1 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 16000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a 4-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **4000 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	1.0 ml	16000 pg/ml
# 1	150 µl of stock	450 µl	4000 pg/ml
# 2	150 µl of 1	450 µl	1000 pg/ml
# 3	150 µl of 2	450 µl	250 pg/ml
# 4	150 µl of 3	450 µl	62.5 pg/ml

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB12)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB12)** 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 10.89 mL of **HRP Diluent Solution** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock

solution 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 of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate 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 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.
5. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 120 minutes on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. 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.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 10-15 minutes on microplate 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.
12. Determine the optical density of each well within 3 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 human soluble IL1RL1 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 for demonstration only. A new standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	CORRECTED (450NM)
Blank	0 (0.082)
62.5	0.041
250	0.229
1000	0.834
4000	2.856

SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Human Soluble IL1R4 Isoform B (HEK293)	100%
Human Soluble (Extracellular Domain) IL1R4 Isoform A (HEK293)	100%
Human Soluble IL1R1	0
Human Soluble IL1R2	0
Human Soluble IL1R3	0
Mouse Soluble IL1R4	0
Human IL-33	0

Human soluble IL1RL1 Isoform B (19K-328F) Fc fusion recombinant derived from NS0 cells and the glycosylated human soluble IL1RL1 Isoform A (19K-328S) derived from HEK293 cells can be detected by this ELISA Kit. The recombinant Human IL1RL1 extracellular domain derived from *E. Coli* or

sf21 expression may NOT be detected by this ELISA Kit.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of Dilution Buffer to blank wells that will be used.
↓
Add 100 µl of standard dilutions, samples, or positive control to each well. Incubate for 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate for 120 minutes 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 plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 10-15 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 3 min.