HUMAN SOLUBLE IL-1 RECEPTOR ANTAGONIST (sIL-1RN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN sIL-1RN CONCENTRATIONS IN SERUM AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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:

ELISA NAME	HUMAN sIL-1RN ELISA
Catalog No.	SK00133-02
Lot No.	
Formulation	96 T
Standard Range	19.5-2500 pg/mL
Sensitivity	9.75 pg/mL
Sample Volume	100 µl
Sample Dilution	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma
Specificity	Human sIL-1RN only
Calibration	Human sIL-1RN Recombinant
Intra-assay Precision	6-8%
Inter-assay Precision	8-12%
Storage	2 – 8 °C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human sIL-1RN ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human sIL-1RN from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human sIL-1RN and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sIL-1RN samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human sIL-1RN. The capture antibody can bind to the human sIL-1RN in the standard and samples. After washing the plate of any unbound substances, an antibody against human sIL-1RN 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 sIL-1RN 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

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_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 with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_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

COMPONENTS PROVIDED

Description	Code	Quantity
sIL-1RN Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against sIL-1RN.	133-02-01	1 plate
sIL-1RN Standard – 5000 pg/vial of recombinant human sIL-1RN in a buffered protein base with preservative; lyophilized.	133-02-02	1 vial
Detection Antibody Concentrate– 1.05 mL / vial, 10-fold concentrate of an antibody against sIL-1RN with preservative; lyophilized.	133-02-03	1 vial
Positive Control - one of recombinant human sIL-1RN; lyophilized.	133-02-04	1 vial
Anti Rabbit IgG-HRP Conjugate -120 µl/vial, 100-fold concentrated solution of Anti-Rabbit IgG conjugate to HRP.	ARIGHRP	1 vial
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	DB08	1 bottle
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	WB01	1 bottle
TMB 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 Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Anti Rabbit IgG-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be store at 2 – 8 °C for up to 6 month (**DO NOT FREEZE** and **PROTECT FROM LIGHT**).

All 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.

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.

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

Serum and plasma samples may not need to be diluted. A pretest will help determine the optimal dilution factor for the samples. **Optimal dilutions should be determined by each laboratory for each application.**
Use polypropylene test tubes.

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.

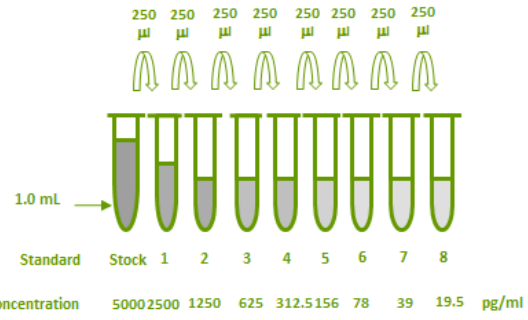
REAGENT PREPARATION

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.

sIL-1RN Standard - Reconstitute the **sIL-1RN** standard with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of the appropriate Dilution Buffer into the tube #1 to #8. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2500 pg/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
Stock	Powder	1000 µl	5000 pg/ml
# 1	250 µl of stock	250 µl	2500 pg/ml
# 2	250 µl of 1	250 µl	1250 pg/ml
# 3	250 µl of 2	250 µl	625 pg/ml
# 4	250 µl of 3	250 µl	312.5 pg/ml
# 5	250 µl of 4	250 µl	156 pg/ml
# 6	250 µl of 5	250 µl	78 pg/ml
# 7	250 µl of 6	250 µl	39 pg/ml
# 8	250 µl of 7	250 µl	19.5 pg/ml



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.

Detection Antibody - 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 the appropriate Dilution Buffer 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 - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. . **Note:** 1x working solution of Anti Rabbit IgG-HRP Conjugate should be used within a few days (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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of Dilution Buffer to Blank wells.
4. Add 100 µL of standard solutions, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 ARIG-HRP Conjugate working solution to each well. Incubate for 1 hour 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 10-20 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

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.085)
19.5	0.029
39	0.055
78	0.091
156	0.150
312.5	0.326
625	0.575
1250	0.945
2500	1.460

- Lot No.:
- Positive Control:

SPECIFICITY

Protein	Cross-reactivity(%)
Human sIL-1RN	100
Human IL-1alpha	0
Human IL-1beta	0
Human sIL-1R II	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 ARIG-HRP conjugate working solution to each well. Incubate 1 hour on the plate shaker at RT. **Protect from light.**

Aspirate and wash 4 times.

Add 100 µl Substrate solution to each well. Incubate 10-20 min on plate shaker at RT. **Protect from light.**

Add 100 µl Stop Solution to each well. Read 450nm within 15 min.