HUMAN SOLUBLE TRAIL R3 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE TRAIL R3 CONCENTRATIONS IN SERUM AND CELL CULTURE SUPERNATES

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE TRAIL R3 ELISA
Catalog No.	SK00253-01
Lot No.	
Formulation	96 T
Standard range	31 - 2000 pg/mL
Sensitivity	10 pg/mL
Sample require	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, Cell Culture Supernates
Specificity	Human TRAIL R3
Intra-assay Precision	4-8%
Inter-assay Precision	6-10%
Storage	2-8°C

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INTRODUCTION

Human Soluble TRAIL R3 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure Human soluble TRAIL R3 in cell culture supernates and serum. It contains recombinant human soluble TRAIL R3 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human soluble TRAIL R3. Results obtained with naturally occurring soluble TRAIL R3 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 soluble TRAIL R3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human soluble TRAIL R3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TRAIL R3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for human soluble TRAIL R3 is added to the wells. Following a wash to remove any unbound antibody 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 human soluble TRAIL R3 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
TRAIL R3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal antibody against soluble TRAIL R3.	**253-01-01**	**1 plate**
TRAIL Standard – 2000 pg/vial of recombinant human soluble TRAIL R3 in a buffered protein base with preservatives; lyophilized.	**253-01-02**	**2 vials**
Detection Antibody – 120 µL/vial, 100-fold Concentrate of Biotinylated antibody against TRAIL R3 with preservatives; lyophilized.	**253-01-03**	**1 vial**
Positive Control – one vial of recombinant human soluble TRAIL R3 , lyophilized	**253-01-04**	**1 vial**
Streptavidin-HRP Conjugate – 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL/vial of buffered protein based solution with preservatives	**DB01**	**1 vial**
Antibody Dilute Solution – 12 mL/vial of buffered protein based solution with preservatives	**DB09**	**1 vial**
Wash Buffer – 50 mL/vial, 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 vial**
TMB Substrate Solution – 11 mL/vial of TMB substrate solution	**TMB01**	**1 vial**
Stop Solution – 11	**S-STOP**	**1 vial**

mL/vial of 0.5M HCL		
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 as well as Dilution Buffer should be stored at -20°C or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin- HRP Conjugate 200-fold Concentrate 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 and seal along entire edge of zip-seal. 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, with the correction wavelength set at 540 nm or 570 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 be 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

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

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 Wash Buffer.

TRAIL R3 Standard - Refer to vial label for reconstitution volume. Reconstitute the **TRAIL R3 Standard** with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 2000 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 #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 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 mL	2000 pg/mL
# 1	250µL of stock	250µL	1000 pg/mL
# 2	250µL of 1	250µL	500 pg/mL
# 3	250µL of 2	250µL	250 pg/mL
# 4	250µL of 3	250µL	125 pg/mL
# 5	250µL of 4	250µL	62.5 pg/mL
# 5	250µL of 5	250µL	31.25 pg/mL

Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 120 µL of Dilution Buffer to produce a 100-fold concentrated stock solution. 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.

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.*

Positive Control - Reconstitute the **Positive Control** with 0.5 mL of Dilution Buffer. *Positive Control should be prepared and used immediately*. *Reconstituted Positive Control CAN NOT BE REUSED*.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of **Dilution Buffer** to Blank well (A2, A3).
4. Add 100 µL of **Standard** (B2, B3 to G2, G3 and G4, G5 to F4, F5), **sample**, or **positive control** (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate 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 **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 micro-plate 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 micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 15-25 minutes 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 micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 TRAIL R3 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.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human TRAIL R3.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of TRAIL R3 was 10 pg/mL.

SPECIFICITY

PROTEIN NAME	CROSS-REACTIVITY
Human Soluble TRAIL R3	100%

Human sTRAIL R1	0
Human sTRAIL R4	0
Human TRAIL	0

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
0 (blank)	(0.092)
31.25	0.029
62.5	0.067
125	0.129
250	0.243
500	0.451
1000	0.907
2000	1.748

- *Lot No.:
- Positive Control:

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µL of **standard, samples, positive control** to each 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 15-25 min on the bench top. **Protect from light.**
↓
Add 100 µl **Stop Solution** to each well. Read 450nm within 15 min