HUMAN SOLUBLE TRAIL /CD253 ELISA KIT

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE TRAIL ELISA
Catalog No.	SK00252-02
Lot No.	
Formulation	96 T
Standard range	62.5 - 4000 pg/mL
Sensitivity	20 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, EDTA Plasma, Cell Culture Supernates
Specificity	Human sTRAIL
Intra-assay Precision	4 - 8%
Inter-assay Precision	6 - 10%
Storage	2 – 8 °C

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

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INTRODUCTION

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for soluble TRAIL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any soluble TRAIL present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for soluble TRAIL 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 soluble TRAIL 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
sTRAIL Microplate – 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against soluble TRAIL.	**252-02-01**	**1 plate**
sTRAIL Standard – 4000 pg/vial of recombinant human soluble TRAIL in a buffered protein base with preservative; lyophilized.	**252-02-02**	**1 vial**
Detection Antibody Concentrate – 1.05mL/vial, 10-fold concentrate of biotinylated antibody against soluble TRAIL with preservative; lyophilized.	**252-02-03**	**1 vial**
Positive Control – one vial of recombinant soluble TRAIL; lyophilized.	**252-02-04**	**1 vial**
Streptavidin-HRP Conjugate – 60 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservative.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB09**	**1 bottle**
Antibody Diluent Solution Concentrate – Lyophilized buffered protein based solution with preservative (11mL).	**DB32**	**1 tube**
Antibody & HRP Diluent Solution – 30 mL 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**
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**
Plastic Pouch	**P01**	**1**

STORAGE

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

Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution, Detection Antibody concentrated solution and Antibody Diluent Solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution (protect from light) and other components may be stored at 2 – 8 °C for up to 8 months. DO NOT FREEZE SAHRP OR TMB SUBSTRATE SOLUTION.

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 after opening.

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

Plasma or serum samples may not require any dilution, but **optimal dilutions should be determined by each laboratory for each application with a pretest.**

Use polypropylene test tubes.

REAGENT PREPARATION

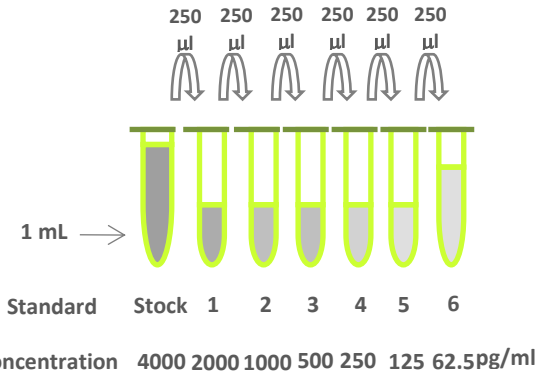
Bring all reagents to room temperature before use.

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.

Antibody Diluent Solution Concentrate – Reconstitute the Antibody Diluent Solution Concentrate with 11.0 mL of **Antibody & HRP Diluent Solution (DB01)** in provided 15 mL centrifuge tube to prepare **Antibody Diluent Solution (DB32)**.

sTRAIL Standard - Refer to vial label for reconstitution volume. Reconstitute the sTRAIL standard with 1.0 mL of **Dilution Buffer (DB09)**. This reconstitution produces a stock solution of 4000 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 (DB09)** into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **4000 pg/mL** standard serves as the high standard. The **Dilution Buffer (DB09)** serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	4000 pg/ml
# 1	250µl of stock	250µl	2000 pg/ml
# 2	250µl of 1	250µl	1000 pg/ml
# 3	250µl of 2	250µl	500 pg/ml
# 4	250µl of 3	250µl	250 pg/ml
# 5	250µl of 4	250µl	125 pg/ml
# 6	250µl of 5	250µl	62.5 pg/ml



Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB32)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of **Antibody Diluent Solution (DB32)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. **Note:** Prepare 1 – 2 hours prior to use.

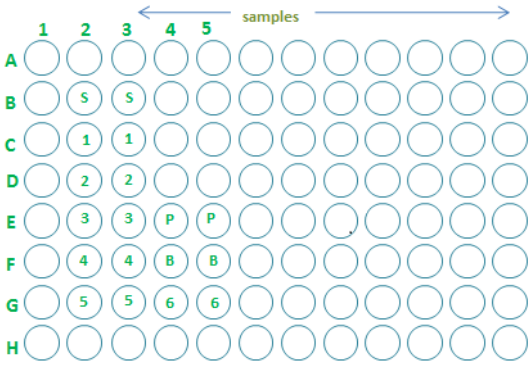
Streptavidin-HRP Conjugate - Transfer 60 µL of 200-fold concentrated stock solution to 11.94 mL of **Antibody & HRP Diluent Solution (DB01)** to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

Positive Control - Reconstitute the positive control with 1.0 mL of **Dilution Buffer (DB09)** to make positive control working solution. **Note:** Positive control working solution 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 (F4, F5).
4. Add 100 µL of standard solutions in reverse order of serial dilutions (G4, G5 and G2, G3 to B2, B3), 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 5-10 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 samples, 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 soluble TRAIL 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 4000 pg/mL may result in inaccurate, low soluble TRAIL levels. Such samples require further external predilution according to expected soluble TRAIL values with Dilution Buffer (DB09) in order to precisely quantify the actual soluble TRAIL level. **Optimal dilutions should be determined by each laboratory for each application.**

CALIBRATION

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

SENSITIVITY

20 pg/mL

TYPICAL DATA

A standard curve should be generated for each set of samples assayed.

SOLUBLE TRAIL (PG/ML)	CORRECTED O.D. (450NM)
Blank	0 (0.089)
62.5	0.041
125	0.086
250	0.158
500	0.302
1000	0.615
2000	1.207
4000	2.223

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human sTRAIL	100%
Human OPG	0
Mouse sTRAIL	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to the wells. Incubate for 2 hours on plate shaker at RT. **Prepare Detection Antibody working solution 1-2 hours prior to use.**
↓
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 Streptavidin-HRP conjugate working solution to each well. Incubate 40 minutes on plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 5-10 minutes on plate shaker at RT. **Protect from light.**

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