

HUMAN SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 1 (TREM-1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF HUMAN SOLUBLE TREM-1
CONCENTRATIONS IN CELL CULTURE
SUPERNATES, SERUM AND PLASMA



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

PURCHASE INFORMATION:

ELISA NAME	HUMAN SOLUBLE TREM-1 ELISA
Catalog No.	SK00218-01
Formulation	96 T
Lot No.	
Standard range	46.875 - 3000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Sample Dilution	Optimal dilutions should be determined by each laboratory for each application.
Sample Type	Cell Culture Supernates, Serum, Plasma
Specificity	Human TREM-1
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	2 - 8 °C

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INTRODUCTION

Human Soluble TREM-1 immunoassay is a solid phase ELISA designed to measure human TREM-1 in cell culture supernates, serum and plasma. It contains recombinant human TREM-1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human TREM-1. Results obtained with naturally occurring TREM-1 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 TREM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TREM-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TREM-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for TREM-1 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 TREM-1 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
TREM-1 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against TREM-1.	218-01-01	1 plate
TREM-1 Standard – 6000 pg/vial of recombinant human TREM-1 in a buffered protein base with preservative; lyophilized.	218-01-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated antibody against TREM-1 with preservative; lyophilized.	218-01-03	1 vial
Positive Control - one vial of recombinant human TREM-1; lyophilized.	218-01-04	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.	DB06	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 solution.	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 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. 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 HRP 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 450nm.
- 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.

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 heparin or EDTA 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. **Optimal dilutions should be determined by each laboratory for each application with a sample pretest.**

Use polypropylene test tubes.

REAGENT PREPARATION

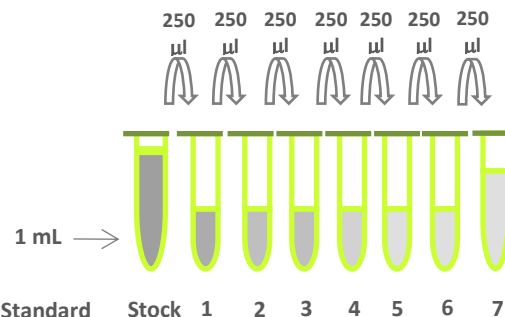
Bring all reagents to room temperature before use.

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.

TREM-1 Standard - Refer to vial label for reconstitution volume. Reconstitute the **TREM-1 Standard** with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 6000 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 #1-6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **3000 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	1000 µl	6000 pg/ml
# 1	250 µl of stock	250 µl	3000 pg/ml
# 2	250 µl of 1	250 µl	1500 pg/ml
# 3	250 µl of 2	250 µl	750 pg/ml
# 4	250 µl of 3	250 µl	375 pg/ml
# 5	250 µl of 4	250 µl	187.5 pg/ml
# 6	250 µl of 5	250 µl	93.75 pg/ml
# 7	250 µl of 6	250 µl	46.875 pg/ml



Concentration 6000 3000 1500 750 375 187.5 93.75 46.875 pg/ml

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 10.8 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 1.2 mL of 10-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

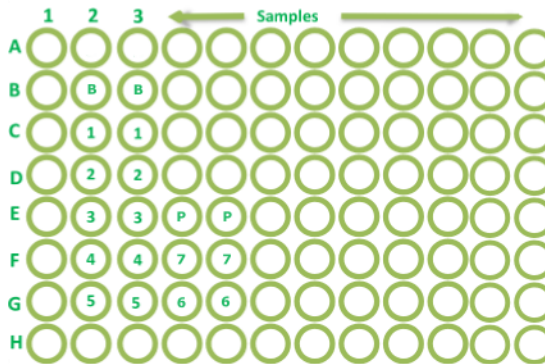
transfer 60 μL of 200-fold concentrated stock solution to prepare working solution. *Note: 1x working solution of Streptavidin-HRP should be used within a few days (protect from light).*

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 $-20\text{ }^{\circ}\text{C}$ or $-70\text{ }^{\circ}\text{C}$.*

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 (B2, B3).
4. Add 100 μL of **Standard solutions** in reverse order of serial dilution (F4, F5 to G4, G5, G2, G3 to C2, C3), **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 1-5 minutes on microplate shaker at room temperature. **Protect from light.**
11. Add 100 μL of **Stop Solution** to each well.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450nm.



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 TREM-1 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 TREM-1.

SENSITIVITY

The minimum detectable dose (MDD) of TREM-1 was 10 pg/mL.

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 O.D. 450NM CORRECTED
Blank	0 (0.067)
46.875	0.031
93.75	0.068
187.5	0.124
375	0.228
750	0.405
1500	0.788
3000	1.483

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human TREM-1	100
Mouse TREM-1	0
Mouse TREM-2b	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 Streptavidin-HRP conjugate working solution to each well. Incubate 45 minutes on the plate shaker at RT. Protect from light.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 4-7 minutes on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 15 minutes.