MOUSE /RAT SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 1 (TREM-1) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE OR RAT 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	MOUSE/RAT SOLUBLE TREM-1 ELISA
Catalog No.	SK00218-03
Formulation	96 T
Lot No.	
Standard range	31-2000 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	Mouse and TREM-1 only
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	2°C-8°C

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

INTRODUCTION

Mouse/Rat Soluble TREM-1 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse or TREM-1 in cell culture supernates, serum and plasma. It contains recombinant mouse TREM-1 and antibodies raised against this protein. It has been shown to accurately quantify recombinant mouse 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 mouse or rat TREM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal 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 polyclonal 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 polyclonal antibody against mouse TREM-1.	**218-03-01**	**1 plate**
TREM-1 Standard – 2000 pg/vial of recombinant mouse TREM-1 in a buffered protein base with preservatives; lyophilized.	**218-03-02**	**1 vial**
Detection Antibody Concentrate – 120 µL/vial, 100-fold concentrated of Biotinylated polyclonal antibody against mouse TREM-1 with preservatives; lyophilized.	**218-03-03**	**1 vial**
Positive Control - one vial of recombinant mouse TREM-1, lyophilized	**218-03-04**	**1 vial**
Streptavidin-HRP Conjugate – 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	**SAHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB06**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservatives	**DB07**	**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	**SDS-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8°C for up to 12 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 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 12 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 after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405 nm and/or 650nm.
- 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.
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.

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.

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 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 #1-6. 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	1000 µl	2000 pg/ml
# 1	250 µl of stock	250 µl	1000 pg/ml
# 2	250 µl of 1	250 µl	500 pg/ml
# 3	250 µl of 2	250 µl	250 pg/ml
# 4	250 µl of 3	250 µl	125 pg/ml
# 5	250 µl of 4	250 µl	62.5 pg/ml
# 6	250 µl of 5	250 µl	31.25 pg/ml

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 120 µL of **Antibody Diluent Solution (DB07)** to produce a 100-fold concentrated stock solution. Pipette 11.88 mL of Antibody Diluent Solution 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 should be used within a few days.*

Positive Control - Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *Note: Positive Control should be prepared and used immediately*.

ASSAY PROCEDURE

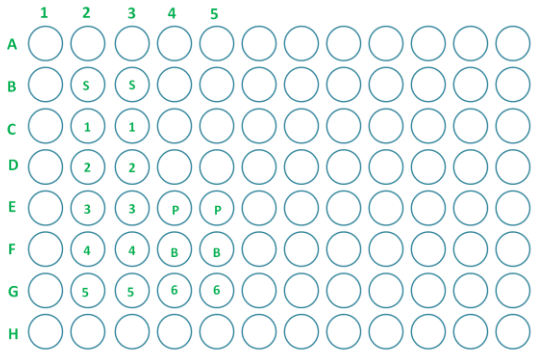
Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.
1. Prepare all reagents and working standards as directed in the previous sections.

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 wells (F4, F5).
4. Add 100 µL of Standard (B2, B3 to G2, G3 and G4, G5), 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 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 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. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of **Substrate Solution** to each well. Incubate for 12-18 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, 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 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.



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 OD405 CORRECTED
Blank	0 (0.073)
31.25	0.040
62.5	0.079
125	0.169
250	0.163
500	0.320
1000	0.682
2000	1.457

- Lot No.:
- Positive Control:
-

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant mouse TREM-1.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of mouse TREM-1 was 10 pg/mL.

SPECIFICITY

The rat serum or plasma samples was detected by mouse TREM1 elisa kit. Its serial diluted samples showed linear curves that were parallel to the standard curves obtained using the kit standards. That results indicated the natural rat serum or plasma samples significant cross-reacts with this ELISA Kit.

PROTEINS	CROSS-REACTIVITY (%)
Mouse TREM-1	100
Human 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 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 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 12-18 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min