

HIGH SENSITIVITY HUMAN TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 2 (TREM-2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF TREM-2 CONCENTRATIONS IN HUMAN SERUM, PLASMA AND RECOMBINANT



ALWAYS REFER TO LOT SPECIFIC PROTOCOL 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:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HIGH SENSITIVITY HUMAN TREM-2 ELISA KIT
Catalog No.	SK00218-12A
Formulation	96 T
Lot No.	20114521
Standard range	31.25 - 2000 pg/ml
Sensitivity	7 pg/ml
Sample Volume	100 µl of diluted samples
Sample Dilution	4- 8 for Serum or Plasma (Optimal dilutions should be determined by each laboratory for each application.)
Sample Type	Serum, Plasma, Recombinant
Specificity	Human TREM-2
Calibration	Human Soluble TREM-2 recombinant (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 6 months. See page 2-3 for detail.
This kit contains sufficient materials to run approximately 40 samples duplicated provided that assay is run according to protocol.	

Order Contact:

AVISCERA BIOSCIENCE, INC.

2348 Walsh Ave., Suite C

Santa Clara, CA 95051

USA

Tel: (408) 982 0300

Email: Sales@AvisceraBioscience.com

Info@AvisceraBioscience.com

www.AvisceraBioscience.com

www.AvisceraBioscience.net

DESCRIPTION

This High Sensitivity Human TREM-2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human soluble TREM-2 from serum, plasma and animal free cell cultures in a sandwich ELISA format.

This immunoassay contains recombinant human soluble TREM-2 from HEK293 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural soluble TREM-2 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human soluble TREM-2. The capture antibody can bind to soluble TREM-2 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human TREM-2 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution is added to the wells and color develops in direct proportion to the amount of human TREM-2 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

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

_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.

_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 cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
sTREM-2 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human sTREM-2.	218-12A-01	1 plate
Human sTREM-2 Standard – 8000 pg/vial of recombinant human sTREM-2 in a buffered protein base with preservative; lyophilized.	218-12A-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of biotinylated antibody against human TREM-2 with preservative; lyophilized.	218-12A-03	1 vial
Positive Control - one vial of recombinant TREM-2; lyophilized.	218-12A-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB32	1 bottle
Antibody & HRP Diluent Solution – 25 mL of buffered protein based solution with preservative.	DB0208C	1 bottle
Wash Buffer - 25 mL of 20-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 0.25M 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 6 months. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Diluent Buffer and Antibody & HRP Diluent Solution concentrate should be stored at - 20° C or -70° C. Streptavidin HRP Conjugate and TMB Substrate Solution should be stored only at 2-8 °C. Do not use kit past expiration date.

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.

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.

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) (Aviscera Bioscience's Order Code: 00700-01-25, 25 TIU for 50 ml sample solution) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

The Serum and plasma samples may require at least a 4 ~8 fold. A suggested 4-fold dilution is 25 µl sample per well + 75 µl Dilution Buffer per well. A suggested 8-fold dilution is 12.5 µl of per well of samples+ 87.5 µl Dilution Buffer per well.

Optimal dilutions should be determined by each laboratory for each application with a sample pretest.

Use polypropylene test tubes.

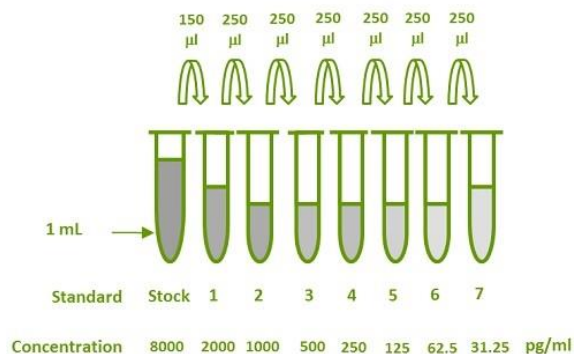
REAGENT PREPARATION

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 **25 mL of Wash Buffer Concentrate (20-fold)** into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

Human sTREM-2 Standard - Reconstitute the sTREM-2 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions. Pipette 250 µL Dilution Buffer into tubes #2-7. Use the high standard (8000 pg/ml) to produce a dilution series (see 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). *Store the stock solution at - 70 °C for a few days*

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	8000 pg/ml
# 1	150 µl of stock	450 µl	2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250 µl of 3	250 µl	250 pg/ml
# 5	250 µl of 4	250 µl	125 pg/ml
# 6	250 µl of 5	250 µl	62.5 pg/ml
# 7	250 µl of 6	250 µl	31.25 pg/ml



Positive Control - Reconstitute the Positive Control with refer to lot specific to prepare the 1x working solution of positive control. Discard the 1x positive control after use. It is for one time use only.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody & HRP Diluent Solution DB0208C** to produce a 10-fold concentrated stock solution.

For 96 wells test, freshly pipette 9.45 mL of **Antibody & HRP Diluent Solution DB0208C** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. For partial strip test, freshly prepare 900 µL per 8-well strip of working solution. Store the stock of 10-fold concentrated solution at -20 °C ~ -70 °C for a few days.

Streptavidin-HRP Conjugate - For 96 wells test freshly pipette 10.89 mL of **Antibody & HRP Diluent Solution DB0208C** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution (*protect from light*). *That 1 x working solution should be used within 20- 30 min.* For partial strip test, freshly prepare 900 µL per 8-well strip of working solution. Store the stock of 100-fold concentrated solution at 2 °C ~ 8 °C for 10 months

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. Add 100 µL per well of **Dilution Buffer** to Blank wells.
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution #7-1, **diluted samples**, or **positive control** per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for 12-17 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of **Stop Solution** to each well.
11. Determine the optical density of each well using a microplate reader set to 450nm within 3 minutes.

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 or 4-parameter curve fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human Soluble TREM-2 (HEK293)	100
Mouse Soluble TREM-2 (HEK293)	0
Human Soluble TREM-1 (HEK293)	0

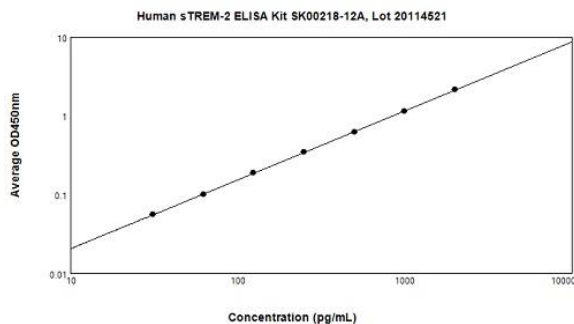
The data indicated the serum samples from goat may have crossreactivity with this ELISA Kit. Recombinant protein samples from HEK293 cells or CHO cells should use animal free cell cultures.

TYPICAL DATA

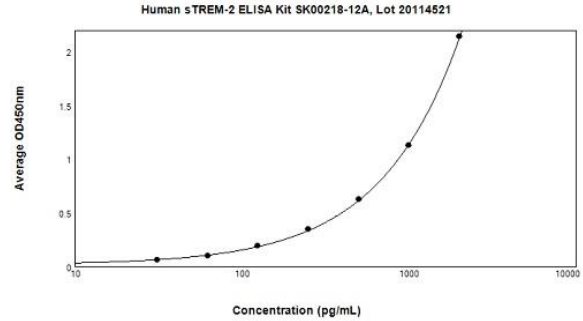
This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450NM CORRECTED
Blank	0 (0.116)
31.25	0.056
62.5	0.099
125	0.186
250	0.342
500	0.624
1000	1.129
2000	2.141

Standard curve by log-log fit:



Standard curve by 4-parameter fit:



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or 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 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 12-17 minutes on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.

Sample Test

The research human samples were diluted by Dilution Buffer DB32. The recovery assayed by Human TREM-2 ELISA Kit SK00218-12A.

Sample	Dilution Factor	Assayed (pg/mL)	Final (ng/mL)	Recovery (%)
Serum	4 X	683.619	2.734	100
Serum	8 X	336.108	2.689	98
Serum	16 X	174.160	2.787	102
Plasma	4 X	402.589	1.610	100
Plasma	8 X	203.156	1.625	101
Plasma	16 X	100.390	1.606	100

The 2 fold diluted samples are detectable. But the recovery are not well.

