

MOUSE/RAT SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS 2 (TREM-2) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION
OF SOLUBLE TREM-2 CONCENTRATIONS IN
MOUSE OR RAT SERUM AND PLASMA



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	MOUSE RAT SOLUBLE TREM-2 ELISA KIT
Catalog No.	SK00218-30
Formulation	96 T
Lot No.	20114542
Standard range	25 - 3200 pg/ml
Sensitivity	10 pg/ml
Sample Volume	100 µl
Sample Dilution	4- 8 for Mouse Serum or Plasma (Optimal dilutions should be determined by each laboratory for each application.)
Sample Type	Serum and Plasma
Specificity	Mouse, Rat TREM-2
Calibration	Mouse 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.	

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DESCRIPTION

This Mouse/Rat Soluble TREM-2 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural mouse or rat soluble TREM-2 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant mouse 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 mouse 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 mouse 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 mouse 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 mouse sTREM-2.	218-30-01	1 plate
Mouse sTREM-2 Standard – 3200 pg/vial of recombinant mouse sTREM-2 in a buffered protein base with preservative; lyophilized.	218-30-02	1 vial
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated antibody against mouse TREM-2 with preservative; lyophilized.	218-30-03	1 vial
Positive Control - one vial of recombinant TREM-2; lyophilized.	218-30-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	SAHRP	1 vial
Sample Diluent Solution – 6 mL of buffered protein based solution with preservative.	DB31	1 bottle
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB12	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB08B	1 bottle
Wash Buffer 20X - 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
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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, **Sample Diluent Solution**, Diluent Buffer and 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). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. Assay samples immediately or aliquot and store 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

Mouse or Rat Serum and plasma samples may need to be diluted by 4-8 fold. **Optimal dilutions should be determined by each laboratory for each application with a sample pretest.**

Use polypropylene test tubes.

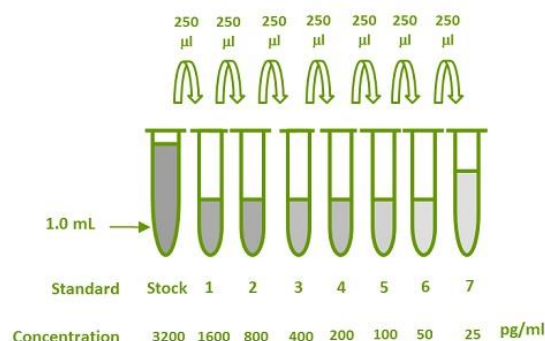
REAGENT PREPARATION

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 X** into deionized or distilled water (**475 mL**) to prepare 500 mL of 1x Wash Buffer.

Mouse sTREM-2 Standard - Reconstitute the Mouse sTREM-2 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 3200 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL Dilution Buffer into tubes #1-7. Use the high standard (3000 pg/ml) to produce a dilution series (see below). Mix each tube thoroughly before the next transfer. The **3200 pg/ml** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Store the stock solution at -20°C ~ -70 °C for a few days

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	3200 pg/ml
# 1	250 µl of stock	250 µl	1600 pg/ml
# 2	250 µl of 1	250 µl	800 pg/ml
# 3	250 µl of 2	250 µl	400 pg/ml
# 4	250 µl of 3	250 µl	200 pg/ml
# 5	250 µl of 4	250 µl	100 pg/ml
# 6	250 µl of 5	250 µl	50 pg/ml
# 7	250 µl of 6	250 µl	25 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer to prepare the 1 x working solution. Discard the 1x positive control after use. It is for one time use only.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.45 mL of Dilution Buffer 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 HRP Diluent Solution (DB08B) 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 8 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 **50 µL per well of Sample Diluent Solution (DB31) to each well**. 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-S, **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 95 minutes 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 17-23 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 5 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 (%)
Mouse Soluble TREM-2 (HEK293)	100
Mouse Soluble TREM-1 (HEK293)	0
Human Soluble TREM-2 (HEK293)	0

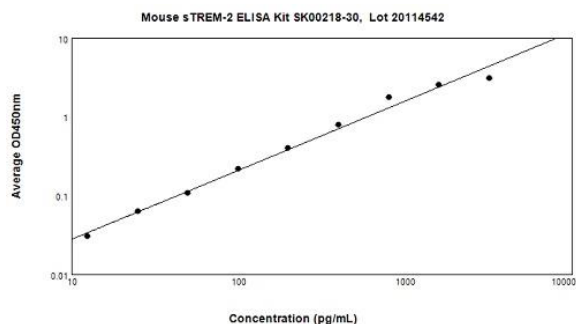
TYPICAL DATA

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

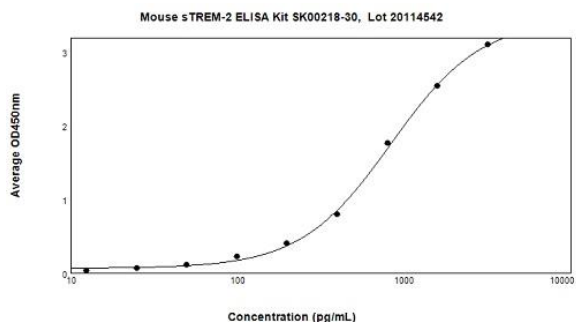
STANDARD (PG/ML)	AVERAGE OD450NM CORRECTED
Blank	0 (0.112)
12.5 (optional)	0.030
25	0.059
50	0.106
100	0.219
200	0.399
400	0.789
800	1.759
1600	2.429
3200	3.014

- Lot No.: 20114542
- Positive Control: 200- 800 pg/mL

STANDARD CURVE BY LOG-LOG:



STANDARD CURVE BY 4-PARAMETER:



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50 µl of Sample Diluent Solution (DB31) to each well. 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 90 min 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 17 ~ 23 minutes on the plate shaker at RT. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 5 min.

Research Samples test:

Add 50µL per well of Sample Diluent Solution DB31 and 100 µl per well of pre-diluted mouse or rat sample solution by Dilution Buffer DB12 or standard solution. Its linearity and recovery was assayed by SK00218-30.

Sample	Dilution Factor	Assayed (PG/mL)	Final (PG/mL)	Recovery (%)
Mouse Plasma	2 X	2118.168	4236.337	100
Mouse Plasma	4 X	1229.601	4918.404	116
Mouse Plasma	8 X	577.924	4623.389	109
Mouse Serum	4 X	1219.338	4877.352	100
Mouse Serum	8 X	612.413	4899.304	100
Rat Plasma	1 X	3458.056	3458.056	100
Rat Plasma	2 X	1966.245	3932.489	114
Rat Serum	1 X	4245.474	4245.474	100
Rat Serum	2 X	2163.058	4326.116	102

Well Position:

