

Quantikine[®] ELISA

Human TREM-1 Immunoassay

Catalog Number DTRM10C

For the quantitative determination of human Triggering Receptor Expressed on Myeloid cells (TREM-1) concentrations in cell culture supernates, cell lysates, serum, plasma, and saliva.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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Manufactured and Distributed by:

USA R&D Systems, Inc.

614 McKinley Place NE, Minneapolis, MN 55413

TEL: 800 343 7475 612 379 2956

FAX: 612 656 4400

E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.

19 Barton Lane, Abingdon Science Park

Abingdon OX14 3NB, UK

TEL: +44 (0)1235 529449

FAX: +44 (0)1235 533420

E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.

Unit 1901, Tower 3, Raffles City Changning Office,

1193 Changning Road, Shanghai PRC 200051

TEL: +86 (21) 52380373 (400) 821-3475

FAX: +86 (21) 52371001

E-MAIL: info.cn@bio-techne.com

INTRODUCTION

TREM proteins (triggering receptor expressed on myeloid cells) are a family of immune regulatory receptors expressed broadly on innate immune cells. TREM proteins are type I transmembrane receptors containing a single immunoglobulin variable (IgV) domain (1-4). These activating and inhibitory receptors are named TREMs and TREM-like transcripts (TLTs), respectively. TREMs have a charged lysine residue in their transmembrane domain that allows for interaction with the ITAM (immunoreceptor tyrosine-based activation motif)-containing transmembrane adapter protein DAP12, which triggers cellular activation (5, 6). In contrast, TLTs lack a transmembrane charged lysine residue but have a long cytoplasmic domain with one or more immunoreceptor tyrosine-based inhibitory motifs (ITIM) that transduce cellular inhibition signals (4).

The 30 kDa mature human TREM-1 consists of a 184 amino acid (aa) extracellular domain, a 29 aa transmembrane domain, and a short 5 aa cytoplasmic domain (5, 7). TREM-1 is expressed by neutrophils, monocytes, and various macrophage subsets (3, 5, 8, 20). During infection by Gram-positive and Gram-negative bacteria and fungi, the expression of membrane-bound TREM-1 is highly upregulated (9, 21). A soluble form of TREM-1 is also released from cells and can be measured in biological fluids (10, 11, 16-18, 20, 21).

TREM-1 is an important regulator in innate immunity and functions to amplify inflammation in response to infection (5, 9, 13, 14). Activation of TREM-1 on monocytes by agonistic antibodies in the presence of TLR (Toll-like receptor) ligands synergistically increases the production of pro-inflammatory chemokines and cytokines and decreases the production of anti-inflammatory IL-10 (12). Monocyte TREM-1 activation also induces the differentiation of primary monocytes into immature dendritic cells (13). On neutrophils, TREM-1 ligation induces degranulation, respiratory burst and phagocytosis synergistically with TLR ligands (14). The production of soluble TREM-1 may distinguish sepsis from non-infectious inflammation (15-18). Furthermore, in mouse models, blockade of TREM-1 can protect against septic shock, suggesting that TREM-1 is a potential target for therapeutic intervention in sepsis in humans (9).

The Quantikine® Human TREM-1 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human TREM-1 levels in cell culture supernates, cell lysates, serum, plasma, and saliva. It contains NS0-expressed recombinant human TREM-1 and antibodies raised against the recombinant protein. Results obtained for naturally occurring human TREM-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this 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 human 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, an enzyme-linked polyclonal antibody specific for human TREM-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, 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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---------------------------|--------|---|--|
| Human TREM-1 Microplate | 894854 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human TREM-1. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| Human TREM-1 Standard | 894856 | 2 vials of recombinant human TREM-1 in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i> | Use a new standard for each assay. Discard after use. |
| Human TREM-1 Conjugate | 894855 | 21 mL of a polyclonal antibody specific for human TREM-1 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| Assay Diluent RD1-27 | 895245 | 11 mL of a buffered protein base with preservatives. | |
| Calibrator Diluent RD5-18 | 895335 | 21 mL of a buffered protein base with preservatives. | |
| Wash Buffer Concentrate | 895003 | 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i> | |
| Color Reagent A | 895000 | 12 mL of stabilized hydrogen peroxide. | |
| Color Reagent B | 895001 | 12 mL of stabilized chromogen (tetramethylbenzidine). | |
| Stop Solution | 895032 | 6 mL of 2 N sulfuric acid. | |
| Plate Sealers | N/A | 4 adhesive strips. | |

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 500 mL graduated cylinder
- Test tubes for dilution of standards and samples
- Human TREM-1 Controls (optional; R&D Systems®, Catalog # QC210)

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- IC Diluent # 12 (R&D Systems®, Catalog # DYC002)
- PBS

PRECAUTIONS

TREM-1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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 or heparin 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: *Citrate plasma has not been validated for use in this assay.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples.

1. Rinse cells with PBS and centrifuge at 2000 x g for 5 minutes to remove supernatant.
2. Solubilize cells at 1×10^7 cells/mL in IC Diluent # 12 and incubate for 15 minutes on ice.
3. Centrifuge at 2000 x g for 5 minutes to remove cell debris.
4. Assay immediately or aliquot the lysis supernates and store at ≤ -20 °C until ready for use.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

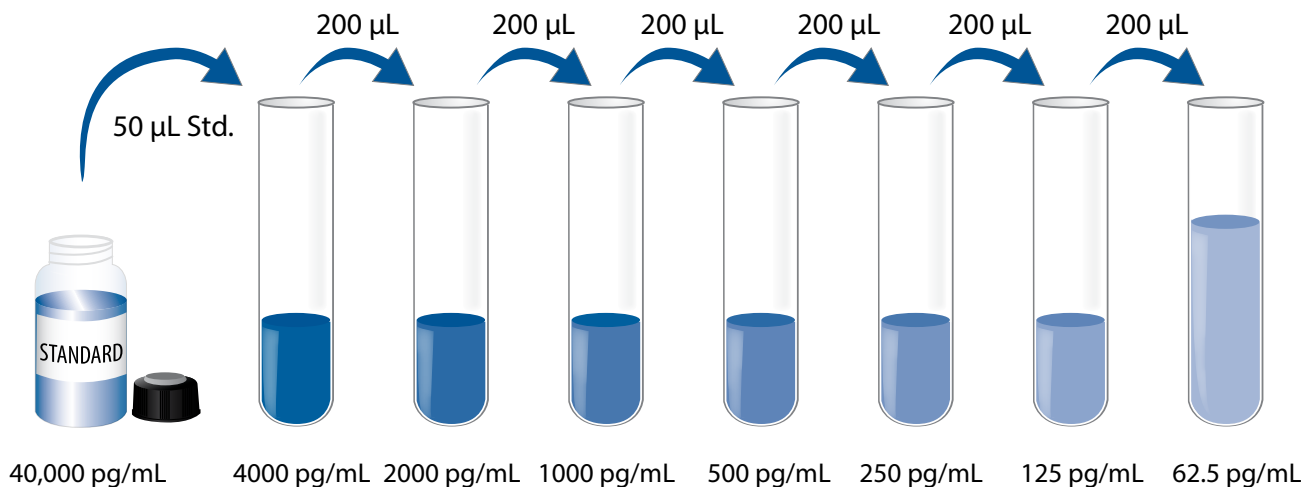
Note: *TREM-1* is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

Human TREM-1 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human TREM-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 μ L of Calibrator Diluent RD5-18 into the 4000 pg/mL tube. Pipette 200 μ L into the remaining tubes. 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. Calibrator Diluent RD5-18 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 μ L of Assay Diluent RD1-27 to each well.
4. Add 50 μ L of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μ L) 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 200 μ L of Human TREM-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 50 μ L 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

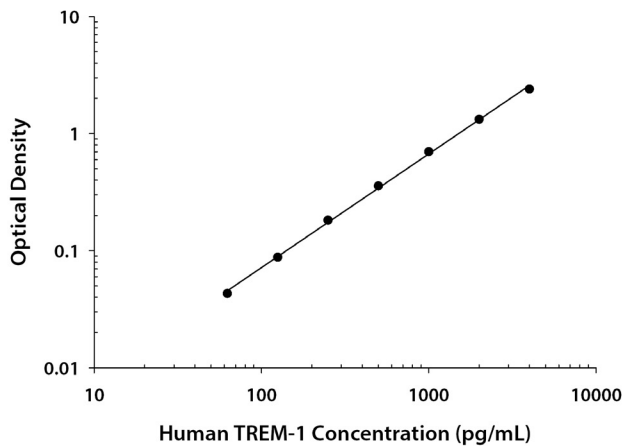
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 a log/log graph. The data may be linearized by plotting the log of the human TREM-1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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.



| (pg/mL) | O.D. | Average | Corrected |
|---------|----------------|---------|-----------|
| 0 | 0.018 0.025 | 0.022 | — |
| 62.5 | 0.064 0.066 | 0.065 | 0.043 |
| 125 | 0.106 0.114 | 0.110 | 0.088 |
| 250 | 0.201 0.206 | 0.204 | 0.182 |
| 500 | 0.376 0.382 | 0.379 | 0.357 |
| 1000 | 0.721 0.722 | 0.722 | 0.700 |
| 2000 | 1.320 1.373 | 1.347 | 1.325 |
| 4000 | 2.418 2.422 | 2.420 | 2.398 |

PRECISION

Intra-Assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)

Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

| Sample | Intra-Assay Precision | | | Inter-Assay Precision | | |
|--------------------|-----------------------|------|------|-----------------------|------|------|
| | 1 | 2 | 3 | 1 | 2 | 3 |
| n | 20 | 20 | 20 | 20 | 20 | 20 |
| Mean (pg/mL) | 479 | 1226 | 2217 | 511 | 1286 | 2254 |
| Standard deviation | 16.8 | 41.5 | 72.1 | 25.0 | 57.7 | 90.7 |
| CV (%) | 3.5 | 3.4 | 3.3 | 4.9 | 4.5 | 4.0 |

RECOVERY

The recovery of human TREM-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|--------------------------|--------------------|---------|
| Cell culture media (n=4) | 102 | 94-112% |
| Serum (n=4) | 97 | 84-107% |
| EDTA plasma (n=4) | 99 | 92-105% |
| Heparin plasma (n=4) | 96 | 87-104% |
| Cell Lysis Buffer (n=4) | 88 | 78-96% |

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human TREM-1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture media (n=4) | Cell lysis buffer (n=3) | Serum (n=4) | EDTA plasma (n=4) | Heparin plasma (n=4) |
|------|-----------------------|--------------------------|-------------------------|-------------|-------------------|----------------------|
| 1:2 | Average % of Expected | 99 | 103 | 108 | 107 | 106 |
| | Range (%) | 95-101 | 99-106 | 105-110 | 105-110 | 103-110 |
| 1:4 | Average % of Expected | 98 | 100 | 108 | 107 | 106 |
| | Range (%) | 95-103 | 90-106 | 105-111 | 103-110 | 102-107 |
| 1:8 | Average % of Expected | 94 | 102 | 99 | 99 | 99 |
| | Range (%) | 90-96 | 91-109 | 98-101 | 94-102 | 97-102 |
| 1:16 | Average % of Expected | 97 | 113 | 104 | 101 | 100 |
| | Range (%) | 86-108 | 109-117 | 94-122 | 94-111 | 94-107 |

SENSITIVITY

Eighteen assays were evaluated and the minimum detectable dose (MDD) of human TREM-1 ranged from 2.65-15.2 pg/mL. The mean MDD was 7.69 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human TREM-1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human TREM-1 in this assay. No medical histories were available for the donors used in this study.

| Sample Type | Mean (pg/mL) | Range (pg/mL) | Standard Deviation (pg/mL) |
|-----------------------|--------------|---------------|----------------------------|
| Serum (n=35) | 277 | 133-471 | 90.0 |
| EDTA plasma (n=35) | 198 | 80.2-382 | 66.0 |
| Heparin plasma (n=35) | 197 | 86.7-404 | 70.1 |

| Sample Type | Mean of Detectable (pg/mL) | % Detectable | Range (pg/mL) |
|---------------|----------------------------|--------------|---------------|
| Saliva (n=10) | 225 | 80 | ND-630 |

ND=Non-detectable

Cell Culture Supernates - Peripheral blood leukocytes were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for human TREM-1.

| Condition | Day 1 (pg/mL) | Day 6 (pg/mL) |
|--------------|---------------|---------------|
| Unstimulated | 185 | 316 |
| Stimulated | 171 | 260 |

Cell Lysates - U937 human histiocytic lymphoma cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cell were lysed according to the Cell Lysis Procedure. An aliquot of the lysate was removed, assayed for human TREM-1, and measured 2514 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human TREM-1.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human TREM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

TREM-2
TREML1

Recombinant mouse:

TREM-1
TREM-2b

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