# ab209881 – Human sTNF R1 (TNFRSF1A) SimpleStep ELISA® Kit

Instructions for use:

For the quantitative measurement of sTNF R1 in human serum, plasma, CSF, urine, and cell culture supernatant.

This product is for research use only and is not intended for diagnostic use.

Version 5 Last Updated 21 August 2026

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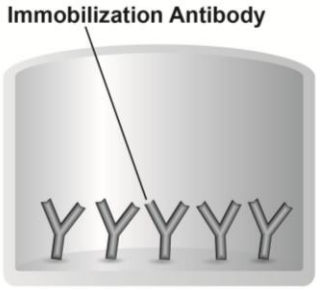
## 1. BACKGROUND

Abcam's sTNF R1 (TNFRSF1A) *in vitro* SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of sTNF R1 protein in human serum, plasma, urine and cell culture supernatants.

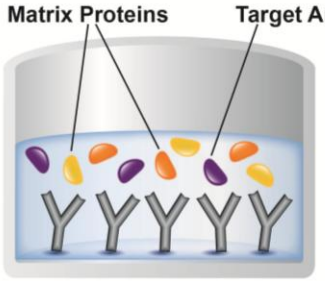
The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Tumor necrosis factor receptor superfamily member 1A (TNF RI) is a transmembrane protein with an extracellular domain that binds to TNF alpha. This extracellular domain can be proteolytically cleaved to make soluble TNF RI. The standard protein in this kit is soluble TNF RI and the capture and detector antibodies were raised against this region of TNF RI.

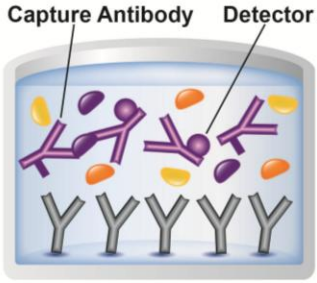
## 2. ASSAY SUMMARY



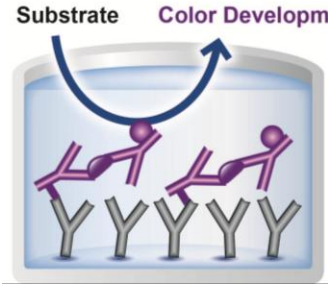

Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all reagents, samples, and standards as instructed.




Add standard or sample to appropriate wells.




Add Antibody Cocktail to all wells. Incubate at room temperature.




Aspirate and wash each well. Add TMB Substrate to each well and incubate. Add Stop Solution at a defined endpoint. Alternatively, record color development kinetically after TMB substrate addition.

## 3. PRECAUTIONS

**Please read these instructions carefully prior to beginning the assay.**

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

## 4. STORAGE AND STABILITY

**Store kit at 2-8ºC immediately upon receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Standard Preparation sections.

## 5. MATERIALS SUPPLIED

| Item | Amount | Storage Condition (Before Preparation) |
|---|---|---|
| 10X Human sTNF R1 Capture Antibody | 600 µL | +2-8ºC |
| 10X Human sTNFR1 Detector Antibody | 600 µL | +2-8ºC |
| Human sTNF R1 Lyophilized Recombinant Protein | 2 Vials | +2-8ºC |
| Antibody Diluent 5BI | 6 mL | +2-8ºC |
| 10X Wash Buffer PT | 20 mL | +2-8ºC |
| TMB Substrate | 12 mL | +2-8ºC |
| Stop Solution | 12 mL | +2-8ºC |
| Sample Diluent NS | 50 mL | +2-8ºC |
| Pre-Coated 96 Well Microplate (12 x 8 well strips) | 96 Wells | +2-8ºC |
| Plate Seal | 1 | +2-8ºC |

## 6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

## 7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.

- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.
- As a guide, typical ranges of sample concentration for commonly used sample types are shown below in Sample Preparation (section 11).
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Incubate ELISA plates on a plate shaker during all incubation steps.
- When generating positive control samples, it is advisable to change pipette tips after each step.
- **To avoid high background always add samples or standards to the well before the addition of the antibody cocktail.**
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

## 9. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. **The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment**.
- Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

### 9.1 1X Wash Buffer PT

Prepare 1X Wash Buffer PT by diluting 10X Wash Buffer PT with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL 10X Wash Buffer PT with 45 mL deionized water. Mix thoroughly and gently.

### 9.2 Antibody Cocktail

Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent 5BI. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent 5BI. Mix thoroughly and gently.

## 10. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

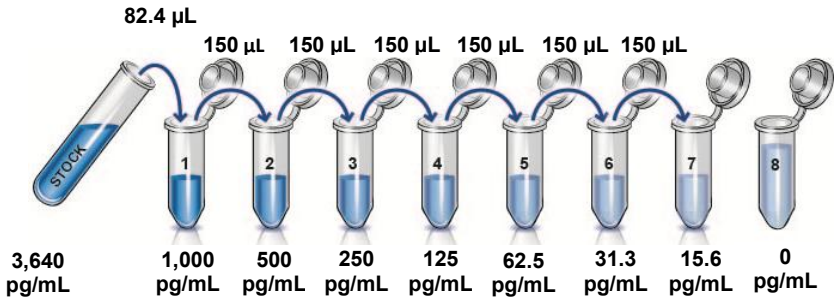
The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the sTNF R1 standard by adding that volume of Sample Diluent NS indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the sTNF R1 standard by adding 500 µL Sample Diluent NS. Hold at room temperature for 10 minutes and mix gently. This is the 3,640 pg/mL **Stock Standard** Solution.

10.2 Label eight tubes, Standards 1– 8.

10.3 Add 217.6 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.

10.4 Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:



## 11. SAMPLE PREPARATION

| TYPICAL SAMPLE DYNAMIC RANGE | |
|---|---|
| **Sample Type** | **Range** |
| Plasma - Heparin | 1:500 – 1:4 |
| Plasma - EDTA | 1:500 – 1:4 |
| Plasma - Citrate | 1:500 – 1:4 |
| Serum | 1:500 – 1:4 |
| Urine | 1:500 – 1:4 |
| Cerebrospinal Fluid | 1:500 – 1:4 |
| PBMC Cell Culture Supernatant | 1%-100% |

11.1 **Plasma**

Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples into Sample Diluent NS and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 **Serum**

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples into Sample Diluent NS and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

11.3 **Cell Culture Supernatants**

Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants and dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.4 **Urine**

Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Collect supernatants, dilute in Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11.5 **Cerebrospinal Fluid (CSF)**

Dilute samples into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

## 12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
- For each assay performed, a minimum of two wells must be used as the zero control.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Differences in well absorbance or “edge effects” have not been observed with this assay.

## 13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4ºC storage.

13.3. Add 50 µL of all sample or standard to appropriate wells.

13.4. Add 50 µL of the Antibody Cocktail to each well.

13.5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

13.6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

13.7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

*Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.*

13.8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

*Alternative to 13.7 – 13.8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:*

| | |
|---|---|
| Mode: | Kinetic |
| Wavelength: | 600 nm |
| Time: | up to 20 min |
| Interval: | 20 sec - 1 min |
| Shaking: | Shake between readings |

*Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.*

13.9. Analyze the data as described below.

## 14. CALCULATIONS

14.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

14.2 **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

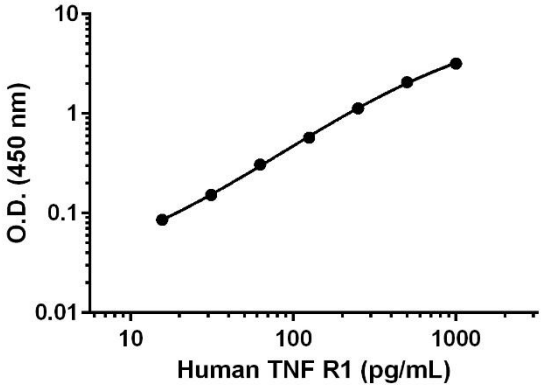
*Note*: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

14.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

14.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

## 15. TYPICAL DATA

**TYPICAL STANDARD CURVE** – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.




| Standard Curve Measurements | | | |
|---|---|---|---|
| Conc. (pg/mL) | O.D. 450 nm | | Mean O.D. |
| | 1 | 2 | |
| 0 | 0.093 | 0.094 | 0.094 |
| 15.6 | 0.183 | 0.177 | 0.179 |
| 31.3 | 0.247 | 0.246 | 0.246 |
| 62.5 | 0.408 | 0.397 | 0.402 |
| 125 | 0.666 | 0.667 | 0.666 |
| 250 | 1.249 | 1.204 | 1.226 |
| 500 | 2.147 | 2.193 | 2.169 |
| 1,000 | 3.296 | 3.286 | 3.291 |

**Figure 1.** Example of human sTNF R1 standard curve. The sTNF R1 standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.

## 16. TYPICAL SAMPLE VALUES

**SENSITIVITY –**

The calculated minimal detectable dose (MDD) is 1.9 pg/mL. The MDD was determined by calculating the mean of zero standard replicates (n=17) and adding 4 standard deviations then extrapolating the corresponding concentration.

**RECOVERY –**

Three concentrations of sTNF R1 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

| Sample Type | Average % Recovery | Range (%) |
|---|---|---|
| 1% Serum | 101 | 87-110 |
| 1% Plasma – Citrate | 98 | 90-105 |
| 1% Plasma – EDTA | 99 | 89-105 |
| 1% Plasma - Heparin | 109 | 105-112 |
| 1% Urine | 107 | 102-116 |
| 12.5% Cerebrospinal Fluid | 116 | 114-117 |
| 25% Cell Culture Media | 108 | 108-109 |

**LINEARITY OF DILUTION –**

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native sTNF R1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

| Dilution Factor | Interpolated value | 6.25% Human Serum | 6.25% Human Plasma-Citrate | 6.25% Human Plasma-EDTA | 6.25% Human Plasma-Heparin |
|---|---|---|---|---|---|
| Undiluted | pg/mL | 148 | 111 | 145 | 157 |
| | **% Expected value** | **100** | **100** | **100** | **100** |
| 2 | pg/mL | 73.7 | 57.5 | 71.4 | 76.7 |
| | **% Expected value** | **99** | **103** | **98** | **96** |
| 4 | pg/mL | 39.3 | 29.6 | 35.6 | 38.5 |
| | **% Expected value** | **106** | **105** | **98** | **98** |
| 8 | pg/mL | 17.7 | 13.9 | 18.9 | 18.9 |
| | **% Expected value** | **96** | **100** | **104** | **96** |
| 16 | pg/mL | 8.05 | 6.56 | 8.26 | 9.60 |
| | **% Expected value** | **87** | **94** | **91** | **98** |

| Dilution Factor | Interpolated value | 6.25% Human Urine | 25% Cerebrospinal Fluid | 50% Unstimulated PBMC Supernatant | 50% Stimulated PBMC Supernatant |
|---|---|---|---|---|---|
| Undiluted | pg/mL | 252 | 523.3 | 44.3 | 96.2 |
| | **% Expected value** | **100** | **100** | **100** | **100** |
| 2 | pg/mL | 125 | 264.5 | 21.4 | 49.3 |
| | **% Expected value** | **99** | **101** | **97** | **103** |
| 4 | pg/mL | 65.3 | 121.7 | 10.5 | 24.9 |
| | **% Expected value** | **103** | **93** | **94** | **104** |
| 8 | pg/mL | 32.9 | 66.6 | 4.74 | 11.2 |
| | **% Expected value** | **104** | **102** | **86** | **93** |
| 16 | pg/mL | 15.6 | 35.5 | 2.32 | 5.07 |
| | **% Expected value** | **99** | **109** | **84** | **84** |

**PRECISION –**

Mean coefficient of variations of interpolated values of sTNF R1 in 3 concentrations of human serum within the working range of the assay.

| | Intra-Assay | Inter-Assay |
|---|---|---|
| n= | 5 | 3 |
| CV (%) | 2.3 | 6.1 |

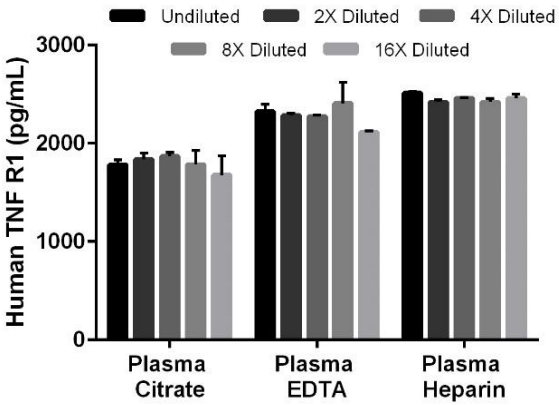



**Figure 2. I**nterpolated concentrations of native sTNF R1 in human plasma samples. The concentrations of sTNF R1 were measured in duplicates, interpolated from the TNF R1 standard curves and corrected for sample dilution. Undiluted samples are as follows: plasma (citrate) 6.25%, plasma (EDTA) 6.25%, and plasma (heparin) 6.25%.The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean sTNF R1 concentration was determined to be 1,791 pg/mL in plasma (citrate), 2,282 pg/mL in plasma (EDTA) and 2,455 pg/mL in plasma (heparin).

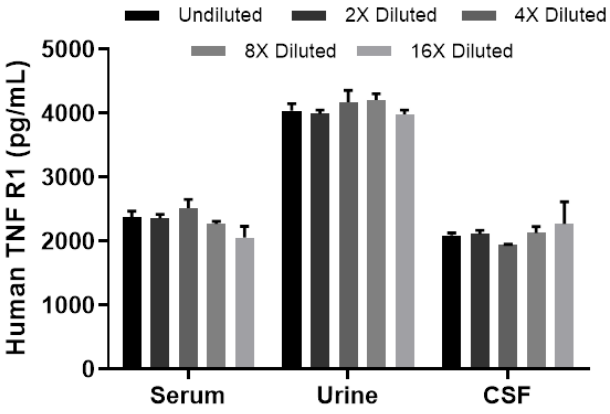



**Figure 3.** Interpolated concentrations of native sTNF R1 in human serum, urine, and CSF samples. The concentrations of sTNF R1 were measured in duplicates, interpolated from the sTNF R1 standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 6.25%, urine 6.25%, and CSF 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean sTNF R1 concentration was determined to be 2,318 pg/mL in serum, 4,082 pg/mL in urine, and 2,112 pg/mL in CSF.

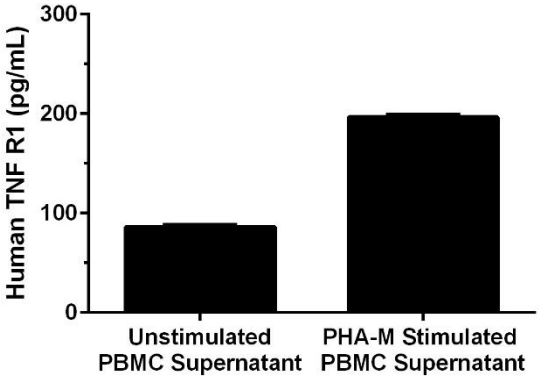



**Figure 4.** Peripheral Blood Mononuclear Cells (PBMC) were grown in the absence (unstimulated) or presence of phytohemagglutinin M (PHA-M) for 2 days. Human sTNF R1 was measured in 2-fold diluted cell culture supernatants of unstimulated and PHA-M stimulated PBMC and cell culture media. Measured values were interpolated from the sTNF R1 standard curve diluted in Sample Diluent NS and corrected for dilution factor. Mean of the duplicate values +/- SD are graphed: 86.1 pg/mL unstimulated, 196.4 pg/mL stimulated and undetectable in media.

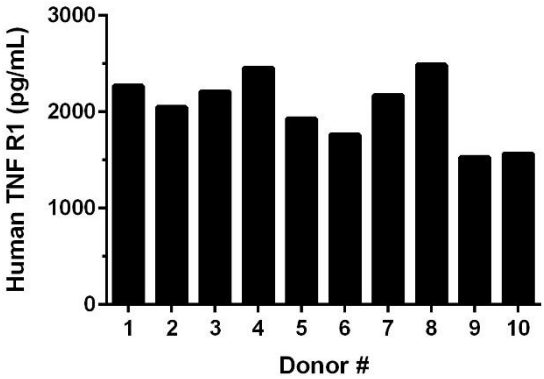



**Figure 5.** Serum from ten individual healthy female human donors was measured in duplicate. Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean sTNF R1 concentration was determined to be 2,043 pg/mL with a range of 1,500 – 2,509 pg/mL

## 17. ASSAY SPECIFICITY

This kit recognizes both native and recombinant human sTNF R1 protein in serum, plasma, CSF, urine, and cell culture supernatant only.

Cell and tissue extract samples have not been tested with this kit.

### CROSS REACTIVITY

Recombinant mouse sTNF R1 and mouse sTNF R2 were prepared at 50 ng/mL and 1 ng/mL and assayed for cross reactivity. No cross-reactivity was observed.

### INTERFERENCE

Recombinant human TNF alpha and mouse TNF alpha were prepared at 1 ng/mL and tested for interference. No interference was observed.

## 18. SPECIES REACTIVITY

This kit recognizes human sTNF R1 protein.

Other species reactivity was determined by measuring 25% serum samples of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Mouse
- Rat
- Cow

Please contact our Technical Support team for more information.

## 19. TROUBLESHOOTING

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | Inaccurate Pipetting | Check pipettes |
| | Improper standard dilution | Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing |
| Low Signal | Incubation times too brief | Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation |
| | Inadequate reagent volumes or improper dilution | Check pipettes and ensure correct preparation |
| | Incubation times with TMB too brief | Ensure sufficient incubation time until blue color develops prior addition of Stop solution |
| Large CV | Plate is insufficiently washed | Review manual for proper wash technique. If using a plate washer, check all ports for obstructions. |
| | Contaminated wash buffer | Prepare fresh wash buffer |
| Low sensitivity | Improper storage of the ELISA kit | Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light. |
| Precipitate in Diluent | Precipitation and/or coagulation of components within the Diluent. | Precipitate can be removed by gently warming the Diluent to 37ºC. |

## 20. NOTES

## Technical Support

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### For all technical or commercial enquiries please go to:

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