

# Human TNF $\alpha$ ELISA Kit

**EH3TNFA EH3TNFA2 EH3TNFA5**

1337.6

| <b>Number</b>   | <b>Description</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>EH3TNFA</b>  | <b>Human Tumor Necrosis Factor <math>\alpha</math> (TNF<math>\alpha</math>) ELISA Kit</b> , sufficient reagents for 96 determinations<br><b>Kit Contents:</b><br><b>Anti-Human TNF<math>\alpha</math> Precoated 96-well Strip Plate</b> , 1 each<br><b>Lyophilized Recombinant Human TNF<math>\alpha</math> Standard</b> , 2 vials<br><b>Sample Diluent</b> , 26 ml, contains 0.1% sodium azide<br><b>Biotinylated Antibody Reagent</b> , 13 ml, contains 0.1% sodium azide<br><b>30X Wash Buffer</b> , 50 ml<br><b>Streptavidin-HRP Reagent</b> , 14 ml<br><b>TMB Substrate</b> , 13 ml<br><b>Stop Solution</b> , 13 ml, contains 0.16 M sulfuric acid<br><b>Adhesive Plate Covers</b> , 6 each                                                                               |
| <b>EH3TNFA2</b> | <b>Human Tumor Necrosis Factor <math>\alpha</math> (TNF<math>\alpha</math>) ELISA Kit</b> , sufficient reagents for 2 $\times$ 96 determinations<br><b>Kit Contents:</b><br><b>Anti-Human TNF<math>\alpha</math> Precoated 96-well Strip Plate</b> , 2 each<br><b>Lyophilized Recombinant Human TNF<math>\alpha</math> Standard</b> , 4 vials<br><b>Sample Diluent</b> , 2 $\times$ 13 ml, contains 0.1% sodium azide<br><b>Biotinylated Antibody Reagent</b> , 2 $\times$ 13 ml, contains 0.1% sodium azide<br><b>30X Wash Buffer</b> , 2 $\times$ 50 ml<br><b>Streptavidin-HRP Reagent</b> , 2 $\times$ 14 ml<br><b>TMB Substrate</b> , 2 $\times$ 13 ml<br><b>Stop Solution</b> , 2 $\times$ 13 ml, contains 0.16 M sulfuric acid<br><b>Adhesive Plate Covers</b> , 12 each |
| <b>EH3TNFA5</b> | <b>Human TNF<math>\alpha</math> ELISA Kit</b> , sufficient reagents for 5 $\times$ 96 determinations<br><b>Kit Contents:</b><br><b>Anti-Human TNF<math>\alpha</math> Precoated 96-well Strip Plate</b> , 5 each<br><b>Lyophilized Recombinant Human TNF<math>\alpha</math> Standard</b> , 5 vials<br><b>Sample Diluent</b> , 75 ml, contains 0.1% sodium azide<br><b>Biotinylated Antibody Reagent</b> , 56 ml, contains 0.1% sodium azide<br><b>30X Wash Buffer</b> , 200 ml<br><b>Streptavidin-HRP Reagent</b> , 70 ml<br><b>TMB Substrate</b> , 5 $\times$ 13 ml<br><b>Stop Solution</b> , 55 ml, contains 0.16 M sulfuric acid<br><b>Adhesive Plate Covers</b> , 30 each                                                                                                   |

For research use only – not for use in diagnostic procedures.

**Storage:** For maximum stability, store in a non-defrosting -20°C freezer and refer to the expiration date for frozen storage on the label. Alternatively, store at 2-8°C and refer to the expiration date for refrigerated storage. Once thawed, store at 4°C until the expiration date for refrigerated storage. Kit is shipped on dry ice.


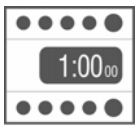


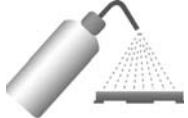







## Introduction

The Thermo Scientific Human TNF $\alpha$  ELISA is an enzyme-linked immunosorbent assay for measuring human TNF $\alpha$  in serum; EDTA, heparin and sodium citrate plasma; and culture supernatants.

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## Procedure Summary

|                                                                                                                                                                                                         |                                                                                                                                                                 |                                                                                                                                                   |                                                                                                                                                                                                        |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <br><b>1.</b> Add 50 $\mu$ l of Sample Diluent to wells. Add 50 $\mu$ l of Standards or Samples to wells in duplicate. | <br><b>2.</b> Cover plate and incubate at room temperature (RT) for 1 hour.    | <br><b>3.</b> Wash plate THREE times.                           | <br><b>4.</b> Add 100 $\mu$ l of Biotinylated Antibody Reagent to wells. Cover plate and incubate at RT for 1 hour. |
| <br><b>5.</b> Wash plate THREE times.                                                                                | <br><b>6.</b> Add 100 $\mu$ l of Streptavidin-HRP Reagent to each well.      | <br><b>7.</b> Cover and incubate plate at RT for 30 minutes.  | <br><b>8.</b> Wash plate THREE times.                                                                             |
| <br><b>9.</b> Add 100 $\mu$ l TMB Substrate to each well.                                                            | <br><b>10.</b> Develop plate at room temperature in the dark for 30 minutes. | <br><b>11.</b> Add 100 $\mu$ l of Stop Solution to each well. | <br><b>12.</b> Measure absorbance and calculate results.                                                          |

## Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1,000  $\mu$ l and plastic pipettes to deliver 5-15 ml
- Ultrapure water for Wash Buffer and Standard reconstitution
- A glass or plastic 2 liter container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5 ml polypropylene or polyethylene tubes to prepare standards – do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- A standard ELISA reader for measuring absorbance at 450 nm and 550 nm. If a 550 nm filter is not available, the absorbance may be read at 450 nm only. Refer to the instruction manual supplied with the instrument being used.
- Graph paper or a computerized curve-fitting statistical software package

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## Precautions

- All specimens and reagents must be at room temperature (20-25°C) before use in the assay.
- Review all instructions carefully and verify components against the Kit Contents list (page 1) before beginning the assay.
- Do not use a 37°C water bath to thaw samples. Thaw samples at room temperature.
- If using a multichannel pipettor, always use a new disposable reagent reservoir for the addition of each reagent. Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Avoid microbial contamination of reagents.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different kit lots. Discard unused ELISA components after completing the assay.
- Do not use glass pipettes to measure the TMB Substrate Solution. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

## Additional Precautions for the 5-plate Kit

- Dispense and equilibrate to room temperature only the reagent volumes required for the number of plates being used. Do not combine leftover reagents with those reserved for additional plates.
- Use only one bottle of the TMB Substrate Solution per 96-well plate. Do not combine leftover substrate with that reserved for other plates.
- Use only one vial of Standard per 96-well plate.

## Sample Preparation

- Serum; EDTA, heparin and sodium citrate plasma; and culture supernatant may be tested in this assay; 50 µl per well of serum, plasma or culture supernatant is required.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- Samples and standards must be assayed in duplicate each time the assay is performed.
- Equilibrate samples gradually to room temperature before beginning the assay. Do not use a heated water bath to thaw or warm samples.
- Mix samples by gently inverting the tubes.
- If samples are clotted, grossly hemolyzed, lipemic or contaminated, make a note on the template and interpret results with caution.
- If the TNFα concentration of a sample possibly exceeds the highest point of the standard curve (i.e., 1,000 pg/ml), prepare one or more 5-fold dilutions of the sample. Prepare a 5-fold dilution by adding 50 µl of sample to 200 µl of Sample Diluent and mix thoroughly. Prepare all sample dilutions using the Sample Diluent provided.

## Reagent Preparation

For procedural differences when running partial plates, look for **(PP)** throughout these instructions.

### Wash Buffer

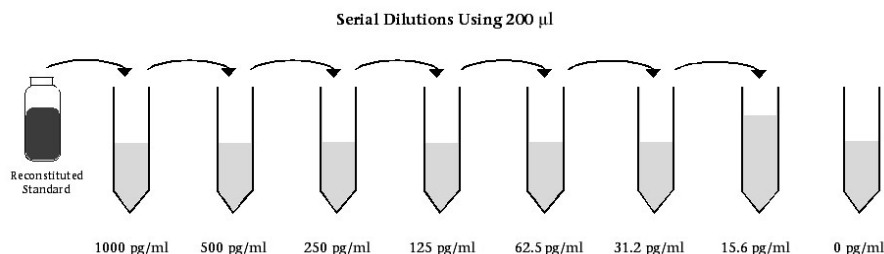
**Note:** Wash Buffer must be at room temperature before use. Do not use Wash Buffer if it becomes visibly contaminated.

1. Label a clean glass or plastic 2 liter container "Wash Buffer." The 30X Wash Buffer may have a cloudy appearance.
2. If using a 5-plate kit, add 30 ml of Wash Buffer to 870 ml of water for each plate; otherwise, add the entire contents of the 30X Wash Buffer (50 ml) bottle to the container and dilute to a final volume of 1.5 L with ultrapure water. Mix thoroughly.

**(PP)** When using partial plates, store the reconstituted Wash Buffer at 2-8°C.

## Standards

- **(PP)** Reconstitute and use one vial of the lyophilized Standard per partial plate.
  - Prepare Standards just before use and use within the same day as reconstitution. Do not store reconstituted standards.
  - Reconstitute Standard in ultrapure water. The reconstitution volume is stated on the standard vial label. Mix by gently inverting the vial until the contents have completely dissolved.
  - When testing both **serum and cell culture supernatant samples** on the same plate, validate the culture media to establish if the same standard curve can be used for both sample types. Prepare a standard curve (including a zero/blank) using culture medium to reconstitute and dilute the standard. Perform this curve in parallel with a standard curve reconstituted in ultrapure water and diluted in the Sample Diluent provided. If OD values are within 10% of the mean for both curves, then either curve may be used.
1. Label seven tubes, one for each standard curve point: 1,000; 500; 250; 125; 62.5; 31.2; 15.6; and 0 pg/ml. Prepare 1:2 serial dilutions to generate the standard curve points as follows:
  2. Pipette 200  $\mu$ l of Sample Diluent into each tube.
  3. Pipette 200  $\mu$ l of the reconstituted Standard into the first tube (1,000 pg/ml) and mix.
  4. Pipette 200  $\mu$ l of this dilution into the next tube (500 pg/ml) and mix.
  5. Repeat serial dilutions (using 200  $\mu$ l) five more times to complete the standard curve points.



## Assay Procedure

### A. Sample Incubation

- **(PP)** Determine the number of strips required and leave these strips in the plate frame. Tightly seal the remaining unused strips in the foil pouch with the desiccant provided and store at 2-8°C. After completing the assay, retain the plate frame for the second partial plate. When using the second partial plate, place the reserved strips securely in the plate frame.
  - Use the Data Template provided to record locations of standards and test samples. Seven standards and one zero must be assayed in duplicate with each series of unknown samples.
  - If the TNF $\alpha$  concentration in any test sample is expected to exceed the highest point on the standard curve (1,000 pg/ml) refer to the Sample Dilution section.
1. Add 50  $\mu$ l Sample Diluent to each well.
  2. Add 50  $\mu$ l standard or sample to each well in duplicate. Mix well by gently tapping the plate several times.
  3. Carefully cover plate with an adhesive plate cover. Ensure that all edges and strips are sealed tightly by running your thumb over the edges and down each strip. Incubate for one (1) hour at room temperature, 20-25°C.
  4. Carefully remove the adhesive plate cover and wash plate as described in the Plate Washing section below.

### B. Plate Washing

1. Gently squeeze the long sides of plate frame before washing to ensure all strips securely remain in the frame.
2. Empty plate contents. Use a squirt bottle to vigorously fill each well completely with Wash Buffer, then empty plate contents. Repeat procedure two additional times for a total of THREE washes. Blot plate onto paper towels or other absorbent material.

**Note:** For automated washing, aspirate all wells and wash THREE times with Wash Buffer, over-filling wells with Wash Buffer. Blot plate onto paper towels or other absorbent material.

### C. Biotinylated Antibody Reagent Incubation

- If using a multichannel pipettor, use a new reagent reservoir to add the Biotinylated Antibody Reagent.
  - **(PP)** Remove from the vial only the amount required for the number of strips being used.
1. Add 100  $\mu$ l of the Biotinylated Antibody Reagent to each well.
  2. Carefully cover plate with an adhesive plate cover. Ensure all edges and strips are tightly sealed by running your thumb over the edges and down each strip. Incubate for one (1) hour at room temperature, 20-25°C.
  3. Carefully remove the adhesive plate cover. Wash plate as described in the Plate Washing section.

### D. Streptavidin-HRP Reagent Incubation

- If using a multichannel pipettor, use new reagent reservoir and pipette tips when adding the Streptavidin-HRP Reagent.
  - **(PP)** Remove from the vial only the reagent amount required for the number of strips being used.
1. Add 100  $\mu$ l of Streptavidin-HRP Reagent to each well.
  2. Carefully attach a new adhesive plate cover, ensuring all edges and strips are tightly sealed. Incubate plate for 30 minutes at room temperature, 20-25°C.
  3. Carefully remove the adhesive cover and discard plate contents. Wash plate as described in the Plate Washing section.

### E. Substrate Incubation and Stop Step

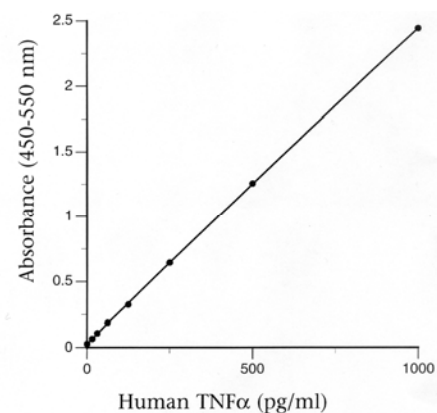
- Use new disposable reagent reservoirs and pipette tips when adding the TMB Substrate Solution and Stop Solution.
  - Dispense from the bottle **ONLY** the amount required for the number of strips being used, 100  $\mu$ l per well. Do not use a glass pipette to measure the TMB Substrate Solution.
  - **(PP)** Do not combine leftover substrate with that reserved for strips or plates not being used. Take care not to contaminate the remaining TMB Substrate Solution.
1. Pipette 100  $\mu$ l of TMB Substrate Solution into each well.
  2. Allow enzymatic reaction to develop at room temperature in the dark for 30 minutes. **Do not cover plate with aluminum foil or a plate sealer.** The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
  3. After 30 minutes, stop the reaction by adding 100  $\mu$ l of Stop Solution to each well.

### F. Absorbance Measurement

- The plate must be evaluated within 30 minutes of stopping the reaction. Measure absorbance on an ELISA plate reader set at 450 nm and 550 nm. Subtract 550 nm values from at 450 nm values to correct for optical imperfections in the microplate. If 550 nm is not available, measure absorbance at 450 nm only. Omitting the 550 nm measurement will result in higher absorbance values.

### G. Calculation of Results

- Generate the standard curve by plotting the average absorbance (450 nm minus 550 nm) obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding TNF $\alpha$  concentration on the horizontal (X) axis.
- Calculate results manually using graph paper or with a curve-fitting statistical software package. If using curve-fitting software, plot a four-parameter logistic curve fit. Alternatively, a point-to-point curve fit may be used. Determine the amount of TNF $\alpha$  in each sample by interpolating from the TNF $\alpha$  concentration (X axis) to the absorbance value (Y axis).
- If the sample was diluted, multiply the interpolated value obtained by the dilution factor to determine amount of TNF $\alpha$  in the sample.
- Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.



## Performance Characteristics

### Sensitivity: < 2 pg/ml

The sensitivity or Lower Limit of Detection (LLD)<sup>1</sup> was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

### Assay Range: 15.6-1,000 pg/ml

Standard curve points are 0, 15.6, 31.2, 62.5, 125, 250, 500 and 1,000 pg/ml.

**Calibration:** The standard in this ELISA is calibrated to NIBSC Standard 87/650. One (1) pg = one (1) NIBSC pg = 0.04 units NIBSC units.

**Reproducibility:** Assay reproducibility was evaluated in each sample matrix. To determine intra-assay precision, 20 replicates of samples containing two levels of recombinant human TNF $\alpha$  were tested on a single plate (Table 1). To evaluate inter-assay precision, three operators who performed at least three separate assays on more than one day tested samples. Twelve duplicate sample values were used to calculate intra-assay precision data for each level of TNF $\alpha$  (Table 1).

**Table 1.** Intra- and inter-assay precision of the human TNF $\alpha$  ELISA kit.

| Sample                   | Level | Intra-assay Precision |            |        | Inter-assay Precision |            |        |
|--------------------------|-------|-----------------------|------------|--------|-----------------------|------------|--------|
|                          |       | Mean (pg/ml)          | SD (pg/ml) | CV (%) | Mean (pg/ml)          | SD (pg/ml) | CV (%) |
| Serum                    | 1     | 87.5                  | 3.6        | 4.2    | 92.8                  | 4.8        | 5.2    |
|                          | 2     | 369                   | 16.6       | 4.5    | 384                   | 19.2       | 5.0    |
| EDTA Plasma              | 1     | 114                   | 6.7        | 5.9    | 117                   | 8.4        | 7.1    |
|                          | 2     | 436                   | 21.2       | 4.8    | 426                   | 20.2       | 4.7    |
| Citrate Plasma           | 1     | 1.5                   | 7.2        | 6.9    | 112                   | 9.0        | 8.0    |
|                          | 2     | 406                   | 22.2       | 5.5    | 384                   | 31.1       | 7.5    |
| Heparin Plasma           | 1     | 116                   | 4.1        | 3.6    | 116                   | 11.0       | 9.6    |
|                          | 2     | 440                   | 17.1       | 3.9    | 427                   | 31.0       | 7.3    |
| Cell Culture Supernatant | 1     | 170                   | 5.1        | 3.0    | 172                   | 7.8        | 4.6    |
|                          | 2     | 623                   | 19.7       | 3.2    | 622                   | 44.8       | 7.2    |

**Specificity:** The following cytokines, tested at 1 mg/ml, did not interfere with or cross-react in the human TNF $\alpha$  ELISA: human IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-12p40, IL-13, IL-15, IL-16, IL-17, IL-18, TNF $\beta$ , Eotaxin, RANTES, GRO $\alpha$ , GRO $\beta$ , MCP-1, MCP-2, MCP-3, MCP-4, VEGF, GCSF, GMCSF, MIP-1 $\alpha$ , MIP-1 $\beta$ , TGF $\beta$ , IFN $\gamma$ , IFN $\alpha$ ; mouse TNF $\alpha$ ; rat TNF $\alpha$ ; and bovine TNF $\alpha$ . TNF $\alpha$  Receptor types 1 and 2 tested at 40 mg/ml did not interfere in this assay. Recombinant pig TNF $\alpha$  cross-reacted at 100%.

**Expected Values:** Thirteen serum and 15 EDTA, heparin and citrate samples collected from apparently healthy individuals were tested. TNF $\alpha$  levels in 50 of the 58 samples tested were below the assay detection limit of 2 pg/ml (Table 2).

**Table 2.** Expected values of TNF $\alpha$  level from healthy individuals.

| Sample Type           | Mean (pg/ml) | Median (pg/ml) | Range (pg/ml) |
|-----------------------|--------------|----------------|---------------|
| Serum (n=35)          | < 2          | < 2            | 0-1.3         |
| EDTA Plasma (n=35)    | 2.45         | < 2            | 0-17.3        |
| Citrate Plasma (n=8)  | < 2          | < 2            | 0-9.6         |
| Heparin Plasma (n=13) | 2.1          | < 2            | 0-16.6        |

**Recovery:** Pooled serum and plasma samples or tissue culture media (TCM) were spiked with recombinant or natural human TNF $\alpha$ . Endogenous TNF $\alpha$  levels were determined by testing non-spiked samples alongside spiked aliquots of the same samples. Expected values were calculated by adding endogenous TNF $\alpha$  levels to those of the spiked control. Percent recovery was calculated by dividing observed by expected values (Table 3).

**Table 3.** Recovery levels of TNF $\alpha$  from samples spiked with recombinant and natural human TNF $\alpha$ .

| Sample         | n | Recombinant TNF $\alpha$ |                       |              | n | Natural TNF $\alpha$  |                       |              |
|----------------|---|--------------------------|-----------------------|--------------|---|-----------------------|-----------------------|--------------|
|                |   | Mean Expected (pg/ml)    | Mean Observed (pg/ml) | Recovery (%) |   | Mean Expected (pg/ml) | Mean Observed (pg/ml) | Recovery (%) |
| Serum          | 8 | 133.2                    | 126.7                 | 95           | 6 | 749.1                 | 775.4                 | 104          |
|                | 8 | 583.2                    | 562.4                 | 96           |   |                       |                       |              |
| EDTA Plasma    | 8 | 105.4                    | 91.7                  | 87           | 6 | 811.2                 | 722.5                 | 95           |
|                | 8 | 561.3                    | 497.8                 | 89           |   |                       |                       |              |
| Citrate Plasma | 8 | 119.8                    | 111.7                 | 93           | 6 | 751.7                 | 760.7                 | 101          |
|                | 8 | 679.5                    | 613.0                 | 90           |   |                       |                       |              |
| Heparin Plasma | 8 | 130.1                    | 114.1                 | 88           | 6 | 845.9                 | 746.4                 | 88           |
|                | 8 | 631.0                    | 597.3                 | 95           |   |                       |                       |              |
| TCM            | 6 | 431.5                    | 441.9                 | 102          | 6 | 397.0                 | 349.49                | 88           |

**Dilution Linearity:** Serum, plasma or tissue culture media (TCM) were spiked with recombinant or natural human TNF $\alpha$ , serially diluted in Sample Diluent and evaluated. Results for heparin and citrate plasma were similar to those for EDTA plasma. Observed values were compared to expected values to calculate percent recovery and demonstrate the dilution linearity of the assay (Table 4 and 5).

**Table 4.** Dilution linearity of samples spiked with recombinant TNF $\alpha$ .

| Sample        | Dilution | Expected (pg/ml) | Observed (pg/ml) | % Recovery |
|---------------|----------|------------------|------------------|------------|
| Serum         | Neat     | 545.6            | 545.6            | -          |
|               | 1:2      | 272.8            | 279.8            | 103%       |
|               | 1:4      | 136.4            | 146.9            | 108%       |
|               | 1:8      | 68.2             | 64.1             | 94%        |
|               | 1:16     | 34.1             | 32.3             | 95%        |
| EDTA Plasma 1 | Neat     | 678.5            | 678.5            | -          |
|               | 1:2      | 339.2            | 354.7            | 105%       |
|               | 1:4      | 169.6            | 162.0            | 96%        |
|               | 1:8      | 84.8             | 88.0             | 104%       |
|               | 1:16     | 42.4             | 40.2             | 95%        |
| TCM           | Neat     | 415.3            | 415.3            | -          |
|               | 1:2      | 207.6            | 205.5            | 99%        |
|               | 1:4      | 103.8            | 103.0            | 99%        |
|               | 1:8      | 51.9             | 46.2             | 89%        |
|               | 1:16     | 26.0             | 27.3             | 105%       |

**Table 5.** Dilution linearity of samples spiked with natural TNF $\alpha$ .

| Sample        | Dilution | Expected (pg/ml) | Observed (pg/ml) | % Recovery |
|---------------|----------|------------------|------------------|------------|
| Serum 2       | Neat     | 793.6            | 793.6            | -          |
|               | 1:2      | 396.8            | 426.3            | 107%       |
|               | 1:4      | 198.4            | 198.7            | 100%       |
|               | 1:8      | 99.2             | 92.1             | 93%        |
|               | 1:16     | 49.6             | 45.0             | 91%        |
| EDTA Plasma 2 | Neat     | 735.1            | 735.1            | -          |
|               | 1:2      | 367.5            | 386.2            | 105%       |
|               | 1:4      | 183.8            | 189.3            | 103%       |
|               | 1:8      | 91.9             | 84.9             | 92%        |
|               | 1:16     | 45.9             | 45.0             | 98%        |
| TCM           | Neat     | 356.4            | 256.4            | -          |
|               | 1:2      | 178.2            | 191.7            | 108%       |
|               | 1:4      | 89.1             | 89.1             | 100%       |
|               | 1:8      | 44.6             | 39.3             | 88%        |
|               | 1:16     | 22.3             | 20.3             | 91%        |

## Reference

1. In *Immunoassay: A Practical Guide*, Chan and Perlstein, Eds. Academic Press: New York, p71.

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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## Data Templates

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |