# HUMAN SOLUBLE TNF RII ELISA KIT

**FOR THE QUANTITATIVE DETERMINATION OF HUMAN SOLUBLE TNF RII CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM AND PLASMA**

**PURCHASE INFORMATION:**

| ELISA NAME | HUMAN SOLUBLE TNF RII ELISA |
|---|---|
| Catalog No. | SK00221-02 |
| Lot No. | |
| Formulation | 96 T |
| Standard range | 7.8-500 pg/mL |
| Sensitivity | 3.9 pg/mL |
| Sample Volume | 100 µl |
| Sample Type | Serum, Plasma, Cell Culture Supernates |
| Dilution Factor | 20 (Optimal dilutions should be determined by each laboratory for each application) |
| Specificity | Human soluble TNF RII |
| Intra-assay Precision | 4-6% |
| Inter-assay Precision | 8-10% |
| Storage | 2°C - 8°C |

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

**Order Contact:**
**AVISCERA BIOSCIENCE INC.**
**2348 Walsh Ave., Suite C**
**Santa Clara, CA 95051**
**Tel: (408) 982 0300**
**Fax: (408) 982 0301**
**Email: Sales@AvisceraBioscience.com**
**Info@AvisceraBioscience.com**
**www.AvisceraBioscience.com**

## INTRODUCTION

Human soluble TNF RII immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human soluble TNF RII in cell culture supernates, serum and EDTA plasma. It contains recombinant human soluble TNF RII and antibodies raised against this protein. It has been shown to accurately quantify recombinant human soluble TNF RII. Results obtained with naturally occurring soluble TNF RII samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human soluble TNF RII.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for soluble TNF RII has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any soluble TNF RII present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for soluble TNF RII is added to the wells. Following a wash to remove any unbound antibody reagent, a Streptavidin-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of soluble TNF RII bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

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_ 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.

_ It is important that the dilution buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute samples with dilution buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

| DESCRIPTION | CODE | QUANTITY |
|---|---|---|
| **SOLUBLE TNF RII Microplate –** 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against SOLUBLE TNF RII. | **221-02-01** | **1 plate** |
| **SOLUBLE TNF RII Standard –** 1000 pg/vial of recombinant human SOLUBLE TNF RII in a buffered protein base with preservatives; lyophilized. | **221-02-02** | **1 vial** |
| **Detection Antibody Concentrate –** 105 µL/vial, 100-fold Concentrate of biotinylated antibody against SOLUBLE TNF RII with preservatives; lyophilized. | **221-02-03** | **1 vial** |
| **Positive Control –** one vial of recombinant human SOLUBLE TNF RII in a buffered protein base with preservatives; lyophilized. | **221-02-04** | **1 vial** |
| **Streptavidin HRP Conjugate –** 75 µL/vial, 200-fold concentrated solution of Streptavidin HRP Conjugate | **SAHRP** | **1 vial** |
| **Dilution Buffer –** 60 mL of buffered protein based solution with preservatives | **DB01** | **1 bottle** |
| **Antibody Diluent Solution –** 12 mL of buffered protein based solution with preservatives | **DB20** | **1 bottle** |
| **Wash Buffer –** 50 mL of 10-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 0.5M HCl | **S-STOP** | **1 bottle** |
| **Plate Sealer** | **EAPS** | **1 piece** |

## STORAGE

**Unopened Kit:** Store at 2 - 8°C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20°C or -70°C. Do not use kit past expiration date.
**Opened/Reconstituted Reagents**: Reconstituted Standard and Detection Antibody Concentrate solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold Concentrate and other components may be stored at 2 - 8°C for up to 6 months.
**Microplate Wells**: Return unused wells to the plastic pouch containing the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2-8°C.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

## PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

## SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
**Plasma** - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Aliquot and store samples at -20°C to -70°C. Avoid repeated freeze-thaw cycles.
**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.

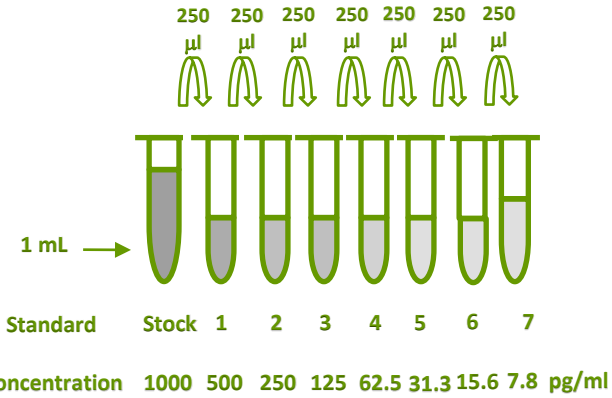
## SAMPLE PREPARATION

Plasma and serum samples may need a 20-fold dilution. An example of a 20-fold dilution is 15µL sample + 385µL of dilution buffer. **Optimal dilutions should be determined by each laboratory for each application.**
**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 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
**SOLUBLE TNF RII Standard - Refer to vial label for reconstitution volume.** Reconstitute the **SOLUBLE TNF RII** standard with 1.0 mL of **Dilution Buffer**. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

| TUBE | STANDARD | DILUTION BUFFER | CONCENTRATION |
|---|---|---|---|
| Stock | powder | 1000 µl | 1000 pg/ml |
| # 1 | 250 µl of stock | 250 µl | 500 pg/ml |
| # 2 | 250 µl of 1 | 250 µl | 250 pg/ml |
| # 3 | 250 µl of 2 | 250 µl | 125 pg/ml |
| # 4 | 250 µl of 3 | 250 µl | 62.5 pg/ml |
| # 5 | 250 µl of 4 | 250 µl | 31.3 pg/ml |
| # 6 | 250 µl of 5 | 250 µl | 15.6 pg/ml |
| # 7 | 250 µl of 6 | 250 µl | 7.8 pg/ml |




**Detection Antibody -** Reconstitute the **Detection Antibody** with 105 µL of Antibody Diluent Solution to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 105 µL of 100-fold concentrated stock solution to prepare working solution. **Note:** Prepare 1-2 hours prior to use.

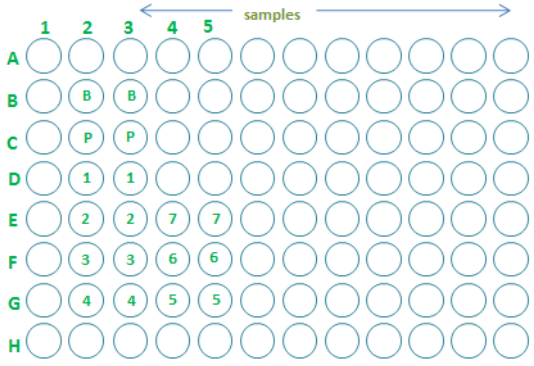
**Streptavidin-HRP Conjugate -** Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

**Positive Control -** Reconstitute the positive control with 2.0mL of Dilution Buffer to make positive control solution.

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that standards and positive control be assayed in duplicates.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to blank wells (B2, B3).
4. Add 100 µL of Standard (from D2, D3 to G2, G3 and E4, E5 to G4, G5), samples, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
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 (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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 5-10 minutes at room temperature. **Protect from light.**
11. Add 100 µL 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.




## 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 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 the graph. The data may be linearized by plotting the log of the SOLUBLE TNF RII concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human SOLUBLE TNF RII.

## SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of SOLUBLE TNF RII was 3.9 pg/mL.

## TYPICAL DATA

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

| SOLUBLE TNF RII (PG/ML) | CORRECTED (450NM) |
|---|---|
| **Blank** | 0 (0.082) |
| **7.8** | 0.047 |
| **15.6** | 0.103 |
| **31.3** | 0.210 |
| **62.5** | 0.367 |
| **125** | 0.774 |
| **250** | 1.461 |
| **500** | 2.645 |

- Lot No:
- Positive Control: 50 – 120 pg/mL

## SPECIFICITY

This assay recognizes both natural and recombinant human SOLUBLE TNF RII. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

| PROTEINS | CROSS-REACTIVITY (%) |
|---|---|
| Human SOLUBLE TNF RII | 100 |
| Human SOLUBLE TNF RI | 0 |
| Human TGF-β | 0 |
| Mouse TNF-α | 0 |
| Mouse SOULBLE TNF RI | 0 |
| Mouse SOLUBLE TNF RII | 0 |

## SUMMARY OF ASSAY PROCEDURE

| PREPARE REAGENTS, SAMPLES AND STANDARDS |
|---|
| ↓ |
| Add 100 µL of standard, samples and positive control to each well. Incubate for 2 hours at room temperature. Prepare Detection Antibody working solution 1-2 hours prior to use. |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µL Detection Antibody working solution to each well. Incubate for 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 min on the plate shaker at RT. **Protect from light.** |
| ↓ |
| Aspirate and wash 4 times. |
| ↓ |
| Add 100 µl Substrate Solution to each well. Incubate 5-10 min on the bench top. **Protect from light.** |
| ↓ |
| Add 100 µl Stop Solution to each well. Read 450nm within 15 min |