# HUMAN SOLUBLE RECEPTOR ACTIVATOR OF NUCLEAR FACTOR-KB (sRANK) ELISA KIT

**For the quantitative determination of human sRANK concentrations in cell culture supernates**

**PURCHASE INFORMATION:**

| ELISA NAME | HUMAN SOLUBLE RANK ELISA |
|---|---|
| Catalog No. | SK00657-01 |
| Lot No. | |
| Formulation | 96 T |
| Standard Range | 62-4000 pg/mL |
| Sensitivity | 31 pg/mL |
| Sample Volume | 100 µl |
| Dilution Factor | Optimal dilutions should be determined by each laboratory for each application |
| Sample Type | Cell Culture Supernates |
| Specificity | Human RANK only |
| Intra-assay Precision | 6 - 8% |
| Inter-assay Precision | 8 - 12% |
| Storage | 2°C - 8°C |

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

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## INTRODUCTION

Human sRANK immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human sRANK in cell culture supernates. It contains recombinant human sRANK and antibodies raised against this protein. It has been shown to accurately quantify recombinant human sRANK. Results obtained with naturally occurring sRANK 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 sRANK.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sRANK has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sRANK present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated polyclonal antibody specific for sRANK is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin 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 sRANK 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 the 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 |
|---|---|---|
| **sRANK Microplate -** 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against RANK. | **657-01-01** | **1 plate** |
| **sRANK Standard –** 4000 pg/vial of recombinant human RANK in a buffered protein base with preservatives; lyophilized. | **657-01-02** | **1 vial** |
| **Detection Antibody Concentrate –** 105 µL/vial, 100-fold concentrated of biotinylated polyclonal antibody against RANK with preservatives; lyophilized. | **657-01-03** | **1 vial** |
| **Positive Control -** one vial of recombinant human RANK, lyophilized | **657-01-04** | **1 vial** |
| **Streptavidin-HRP Conjugate -** 120 ul/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP with preservatives | **SAHRP** | **1 vial** |
| **Dilution Buffer -** 60mL of buffered protein based solution with preservatives | **DB08** | **1 bottle** |
| **Antibody Diluent Solution -** 12 mL of buffered protein based solution with preservatives | **DB28** | **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 as well as Dilution Buffer should be stored at -20°C or -70°C. Do not use kit past expiration date.

**Opened / Reconstituted Reagents:** Reconstituted Standard and Antibody Concentrate Solution SHOULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 100-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 bag 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.

## 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.
**Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.**

## SAMPLE PREPARATION

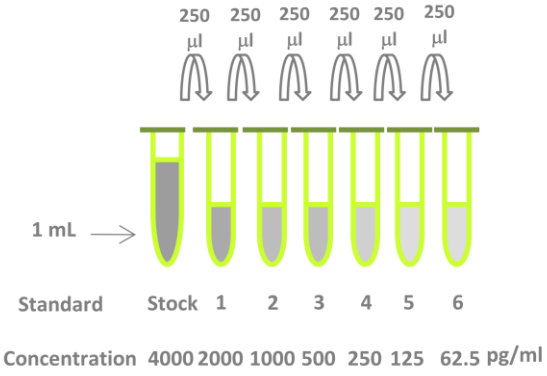
Optimal dilutions should be determined by each laboratory for each application.
**Use polypropylene test tubes.**

## 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.

**RANK Standard - Refer to vial label for reconstitution volume.** Reconstitute the **RANK** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Dilution Buffer into tubes #1 to #6. 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. The Dilution Buffer serves as the zero standard (0 pg/mL).

| Tube | Standard | Dilution Buffer | Concentration |
|---|---|---|---|
| **Stock** | Powder | 1000 µl | 4000 pg/ml |
| **# 1** | 250 µl of stock | 250 µl | 2000 pg/ml |
| **# 2** | 250 µl of 1 | 250 µl | 1000 pg/ml |
| **# 3** | 250 µl of 2 | 250 µl | 500 pg/ml |
| **# 4** | 250 µl of 3 | 250 µl | 250 pg/ml |
| **# 5** | 250 µl of 4 | 250 µl | 125 pg/ml |
| **# 6** | 250 µl of 5 | 250 µl | 62.5 pg/ml |




**Detection Antibody Concentrate -** Reconstitute the Detection Antibody Concentrate with 105 µl of **Antibody Diluent Solution (DB28)** to produce a 100-fold concentrated stock solution. Transfer the 105 µl of 100-fold concentrated stock solution to 10.395 mL of **Antibody Diluent Solution** to prepare working solution. **Note:** Prepare 1 to 2 hours prior to use.

**Streptavidin-HRP Conjugate** - Pipette 11.88 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP conjugate should be used within a few days.

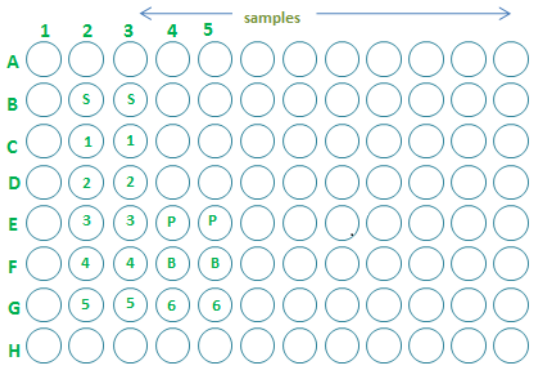
**Positive Control -** Reconstitute the positive control with 1.0 mL of Dilution Buffer to make positive

control solution. **Note:** Positive Control should be used immediately.

## ASSAY PROCEDURE

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

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 (F4, F5).
4. Add 100 µL of **Standard** (B2, B3 to G2, G3 and G4, G5), **sample**, or **positive control** (E4, E5) 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 **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.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with 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 45 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 15-30 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 sRANK 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 RANK/Fc chimera.

## SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of RANK was 31 pg/mL.

## SPECIFICITY

This assay recognizes both natural and recombinant human sRANK. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following

factors at 50 ng/mL in a mid-range rh RANK control were assayed for interference. No significant cross-reactivity or interference was observed.

| Protein | Cross-reactivity (%) |
|---|---|
| **Human RANK/Fc Chimera** | 100 |
| **Mouse RANK/Fc** | 0.4 |
| **Human TRANCE** | 0 |
| **Human OPG/Fc** | 0 |
| **Human OX40/Fc** | 0 |
| **Human 4-1BB** | 0 |

## LINEARITY

To assess the linearity of the assay, pooled research human **plasma and serum** samples were diluted with Dilution Buffer and assayed. However, our samples were not detectable.

We also tested samples spiked with maximum standard concentration. 1 part maximum standard: 3 parts sample (1/4X dilution of maximum standard = 1000 pg/mL)

| 25% SPIKED SAMPLES | ESTIMATED CONCENTRATION (PG/ML) | FINAL (PG/ML) | RECOVERY (%) |
|---|---|---|---|
| **Plasma** | 1000 | 972.848 | 97.2 |
| **Serum** | 1000 | 1514.056 | 151 |

## TYPICAL DATA

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

| STANDARD (PG/ML) | AVERAGE OD450 (CORRECTED)* |
|---|---|
| Blank | 0 (0.045) |
| 62.5 | 0.007 |
| 125 | 0.015 |
| 250 | 0.042 |
| 500 | 0.096 |
| 1000 | 0.191 |
| 2000 | 0.418 |
| 4000 | 0.848 |

- Lot No.:
- Positive Control: 350 – 650 pg/mL

## SUMMARY OF ASSAY PROCEDURE

| PREPARE REAGENTS, SAMPLES AND STANDARDS |
|---|
| ⇩ |
| Add 100 µl of standard, samples, positive control to each 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 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 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 15-30 min on the bench top. **Protect from light.** |
| ⇩ |
| Add 100 µl Stop Solution to each well. Read 450nm within 15 min |