Soluble ST2 (Human)

ELISA KIT PROTOCOL

(Catalog No.: EK-036-10)

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INTRODUCTION

ST2, also known as IL-1 R4 and T1, is an Interleukin-1 receptor family glycoprotein that contributes to Th2 immune responses (1, 2). Human ST2 consists of a 310 amino acid (aa) extracellular domain (ECD) with three Ig-like domains, a 21 aa transmembrane segment, and a 207 aa cytoplasmic domain with an intracellular TIR domain (3, 4). Alternate splicing of the 120 kDa human ST2 generates a soluble 60 kDa isoform that lacks the transmembrane and cytoplasmic regions as well as an isoform that additionally lacks the third Ig-like domain (4). Within the ECD, human ST2 shares 68% and 64% aa sequence identity with mouse and rat ST2, respectively. ST2 is expressed on the surface of mast cells, activated Th2 cells, macrophages, and cardiac myocytes (5 - 8). It binds IL-33, a cytokine that is upregulated by inflammation or mechanical strain in smooth muscle cells, airway epithelia, keratinocytes, and cardiac fibroblasts (5, 9). IL-33 binding induces the association of ST2 with IL-1R AcP, a shared signaling subunit that also associates with IL-1 RI and IL-1 R rp2 (1, 10, 11). In macrophages, ST2 interferes with signaling from IL-1 RI and TLR4 by sequestering the adaptor proteins MyD88 and Mal (7). In addition to its role in promoting mast cell and Th2 dependent inflammation, ST2 activation enhances antigen induced hypernociception and protects from atherosclerosis and cardiac hypertrophy (5, 12 - 14). The soluble ST2 isoform is released by activated Th2 cells and strained cardiac myocytes and is elevated in the serum in allergic asthma (6, 8, 15). Soluble ST2 functions as a decoy receptor that blocks IL-33's ability to signal through transmembrane ST2 (10, 13 - 15).

Phoenix Pharmaceutical's Human Soluble ST2 ELISA Kit is designed to measure the concentration of Human Soluble ST2 from human serum/plasma, or conditioned medium.

PROTOCOL OVERVIEW

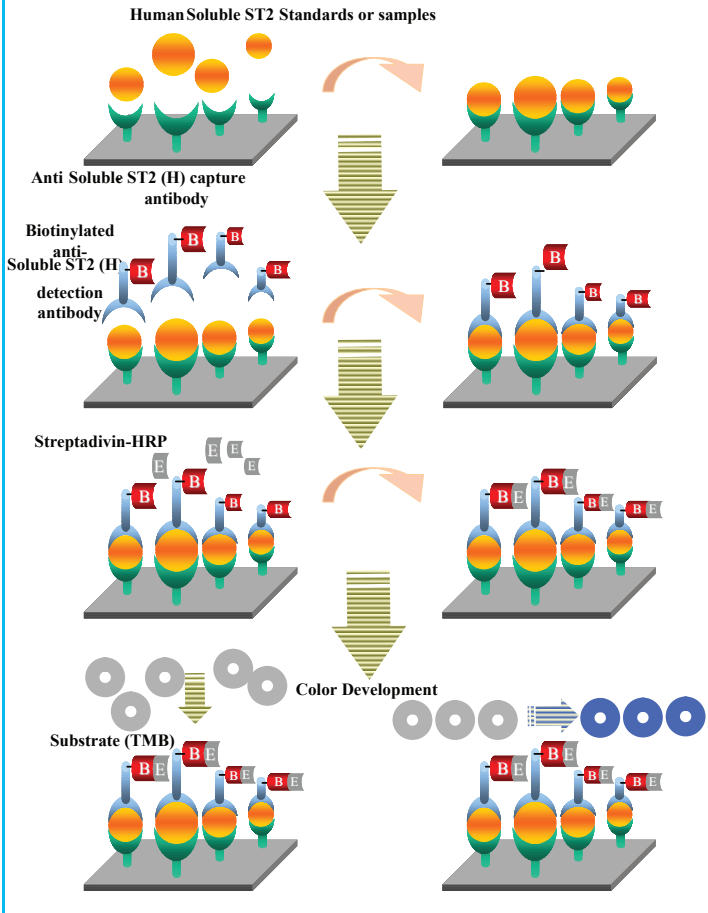
The immunoplate in this kit is pre-coated with anti-Human Soluble ST2 Capture Antibody and the non-specific binding sites are blocked. The Human Soluble ST2 in the sample or in the standard solution can bind to the capture antibody immobilized in the wells. After washing procedure, the Biotinylated anti-Human Soluble ST2 Detection Antibody can bind to the Human Soluble ST2 trapped in the wells is added. After washing, the Streptavidin-Horseradish Peroxidase (SA-HRP) which catalyzes the Substrate Solution (TMB) is added. The enzyme-substrate reaction is terminated by the addition of a stop solution. The intensity of the the color is directly proportional to the amount of Human Soluble ST2 in the standard solutions or samples. A standard curve of Human Soluble ST2 with known concentration can be established accordingly. The Human Soluble ST2 with unknown concentration in samples can be determined by extrapolation to this standard curve.

ASSAY CONDITIONS

Plasma, serum, culture media, tissue homogenate, CSF, urine or any biological fluid can be assayed as long as the level of the sample is high enough the for the sensitivity of the kit to detect it.

Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

ASSAY PRINCIPLE



LIST OF COMPONENTS

Store all components at 4°C. DO NOT FREEZE.

1. **20x** Assay Buffer Concentrate *(50ml)*.....**Catalog No. EK-BUF**
2. **1x** Dilution Buffer*(75ml)*.....**Catalog No. EK-DB-036-10**
3. 96 Well anti-Soluble ST2.....**Catalog No. EK-Plate-036-10** Capture Antibody-Coated Plate *(1 plate)*
4. Human Soluble ST2 Standard.....**Catalog No. EK-S-036-10** *(4,000pg/vial)*
5. Biotinylated anti-Human Soluble ST2.....**Catalog No. EK-D-036-10** Detection Antibody *(1 vial)*
6. Human Soluble ST2 Positive Control.....**Catalog No. EK-PC-036-10** *(2 vials)*
7. Streptavidin-Horseradish..... **Catalog No. EK-SA-HRP** Peroxidase (SA-HRP) *(30µl)*
8. Substrate Solution (TMB) *(12ml)*.....**Catalog No. EK-SS**
9. Stop Solution 2N HCl *(15ml)* .....**Catalog No. EK-HCL**
10. Acetate Plate Sealer (APS) *(3 pieces)*.....**Catalog No. EK-APS**
11. Assay Diagram *(1 sheet)*

MATERIALS REQUIRED BUT NOT SUPPLIED

- Micropipettor(s) and disposable pipette tips
- Multi-channel pipette capable of dispensing 50-100µl
- Solution Reservoir *(recommended)*
- Microtiter plate washer *(recommended)*
- Orbital plate shaker capable of 300-500 rpm *(recommended)*
- Microtiter plate reader capable of absorbance measurement 450nm
- Well-closed containers (15ml tubes or more in capacity)
- Absorbent material for blotting

REAGENT PREPARATION

Note: *The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.*

1. **1x Assay Buffer:** Dilute the **20x** Assay Buffer Concentrate with 950ml of distilled water. This assay buffer will be used to wash the plate in this kit. If crystals are observed in the **20x** Assay Buffer warm the bottle in a 37°C water bath for approximately 30 minutes or until the crystals disappear. After preparation, store **1x** Assay Buffer at 4°C.
2. **1x Dilution Buffer:** Ready to use.
3. **Biotinylated anti-Human Soluble ST2 Detection Antibody:** Rehydrate Biotinylated anti-Human Soluble ST2 Detection Antibody with 200µl of **1x** Dilution Buffer, vortex (centrifuge the tube to dislodge powder from the cap or walls). Dilute Biotinylated anti-Human Soluble ST2 Detection Antibody to 1:60 and mix thoroughly before use.
4. **Streptavidin-Horseradish Peroxidase (SA-HRP):** Centrifuge the HRP vial (30µl) provided in this kit (3,000-5,000 rpm, 5 seconds) and dilute HRP with **1x** Dilution Buffer to 1:2000 before use. Vortex thoroughly.
5. **Human Soluble ST2 Positive Control:** Rehydrate Human Soluble ST2 Positive Control with 250µl of **1x** Dilution Buffer (centrifuge the tube to dislodge powder from cap or walls). Vortex thoroughly.

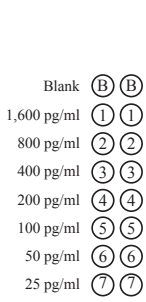
HUMAN SOLUBLE ST2 STANDARD PREPARATION

1. Rehydrate recombinant Human Soluble ST2 Standard with 1ml **1x** Dilution Buffer, vortex. Allow the solution to sit at least 10 minutes at room temperature (20-23°C) to completely dissolve in solution. Vortex and centrifuge before use. The concentration of this stock solution is 4,000pg/ml.

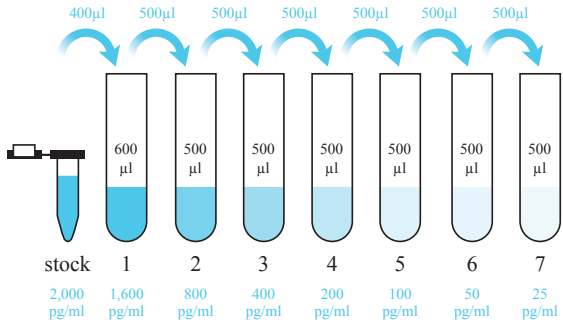
2. Prepare Standard solutions as follows:

Standard No.	Standard Protein Volume	1x Dilution Buffer Volume	Concentration
Stock	Powder	1000µl	4000pg/ml
#1	400µl of Stock	600µl	1600pg/ml
#2	500µl of #1	500µl	800pg/ml
#3	500µl of #2	500µl	400pg/ml
#4	500µl of #3	500µl	200pg/ml
#5	500µl of #4	500µl	100pg/ml
#6	500µl of #5	500µl	50pg/ml
#7	500µl of #6	500µl	25pg/ml

Table of Standard Dilutions



Immunoplate loading map



Visual Guide of the Standard Dilutions

HUMAN SOLUBLE ST2 ELISA PROTOCOL

1. Thoroughly read this protocol before performing an assay. Allow all reagents to come to room temperature (20-23°C) prior to the start of the assay.
2. Remove Capture Antibody-Coated Plate from its zip-lock foil pouch. Remove unneeded strips from the plate frame, reseal them in the foil pouch, and return the foil pouch to 4°*C*.
3. Wash each well with 350µl of **1x** Assay Buffer. Allow it to sit for at least five minutes. Discard the buffer, invert and blot dry plate. Do not let wells dry before proceeding to the next step.
4. Leave wells A-1 and A-2 empty as **Blank**.
5. Add 100µl of the prepared Human Soluble ST2 Standard solutions from **#7** to **#1** (reverse order of serial dilution) in duplicate to each well.
6. Add 100µl of Human Soluble ST2 Positive Control solution in duplicate.
7. Add 100µl diluted samples in duplicate into their designated wells.
8. Seal the immunoplate with Acetate Plate Sealer (APS). Incubate for 2 hours at room temperature (20-23°C) on a plate shaker (300-400 rpm).
9. Before washing the plate, remove the plate sealer carefuly. Completely discard the liquid from wells. Wash each well with 300-350µl assay buffer four times. At the end of each wash, discard the buffer, invert the plate, and tap on a clean absorbent towel.
10. Add 100µl Biotinylated anti-Human Soluble ST2 Detection Antibody into each well. Reseal the immunoplate with plate sealer and incubate for 2 hours at room temperature (20-23°C) on a plate shaker (300-400rpm).
11. Wash 4 times with the **1x** Assay Buffer as described in step 9.
12. Add 100µl SA-HRP solution into each well. Reseal the immunoplate with plate sealer and incubate the plate for 20 minutes at room temperature (20-23°C) on plate shaker (300-400rpm).

13. Wash 4 times with the **1x** Assay Buffer as described in step 9.
14. Add 100µl Substrate Solution (TMB) provided in this kit into each well. Reseal the plate with plate sealer to protect from light and incubate the plate for 20-30 minutes at room temperature (20-23°C) on a plate shaker (300-400 rpm).
15. Add 100µl Stop Solution (2N Hydrochloric Acid) into each well to stop the reaction. The color in the well should change from blue to yellow. If the color change does not appear to be uniform, gently tap the plate to ensure thorough mixing. Proceed to the next step within 20 minutes.
16. Read Absorbance O.D. at 450nm using a Microtiter Plate Reader.

ADDITIONAL RECOMMENDED PROCEDURAL NOTES:

- Reagents of different lot numbers should not be mixed.
- Recheck the reagent labels when loading the plate to ensure that everything is added correctly.
- Unused microplate strips should be placed in the foil pouch with a desiccant and stored at 4°C. Do not allow moisture to enter the wells.
- When handling the plate, avoid touching the bottom.
- Manual washing may cause high duplicate coefficient variations. To reduce this factor, liquid from the plate should be removed by inverting and blotting the plate on an absorbent material.
- If the room temperature is not within the suggested range (20-23°C), variations in results may occur.
- The same reservoir for the reagents may be reused if the reservoir is washed well with distilled water before each use.
- Each laboratory must determine the appropriate dilution factors for the samples to be measured to ensure that the samples are within the dynamic range of the standard curve.
- High levels of interfering proteins may cause variations within the sample results. Therefore, it is imperative to select the appropriate sample preparation procedure to obtain the optimal results.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.
- For optimal results, an orbital plate shaker capable of 300-500 rpm is recommended for all incubations.
- Modification of the existing protocol (i.e. standard dilutions, pipetting technique, washing technique, incubation time or temperature, storage conditions, and kit expiration) may affect the sensitivity and specificity of the test.

SUMMARY OF ASSAY PROTOCOL

Add 100µl/well of Soluble ST2 standard, sample, or positive control **except the Blank wells**

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Incubate at room temperature (20-23°C) for 2 hours

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Wash immunoplate 4 times with 350µl/well of 1x assay buffer

▼

Add 100µl/well of Biotinylated anti-Soluble ST2 Detection Antibody

▼

Incubate at room temperature (20-23°C) for 2 hours

▼

Wash immunoplate 4 times with 350µl/well of **1x** assay buffer

▼

Add 100µl/well of SA-HRP solution

▼

Incubate at room temperature (20-23°C) for 20 minutes

▼

Wash immunoplate 4 times with 350µl/well of **1x** assay buffer

▼

Add 100µl/well of Substrate Solution (TMB)

▼

Incubate at room temperature (20-23°C) for 20-30 minutes

▼

Terminate reaction with 100µl/well of 2N HCL

▼

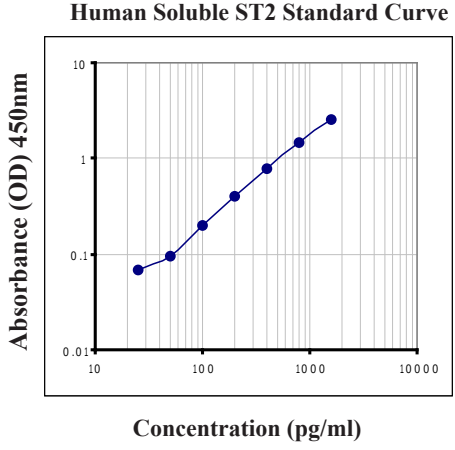
Read absorbance O.D. at **450nm** and calculate results

CALCULATION OF RESULTS

Plot the standard curve on log-log graph paper. Known concentration of Human Soluble ST2 Standard and its corresponding O.D. reading is plotted on the log scale (X-axis) and the log scale (Y-axis), respectively. The standard curve shows a correlated relationship between Human Soluble ST2 concentrations and the corresponding O.D. absorbance. As the standard concentration increases, the intensity of the yellow color, and in turn the O.D. absorbance, increases.

The concentration of Human Soluble ST2 within a sample is determined by plotting the sample's O.D. on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point will intersect the X-axis at a coordinate corresponding to the Human Soluble ST2 concentration in the unknown sample.

Refer to QC Data sheet for acceptable values of the positive conrtol.



STORAGE

1. Store the kit at 4°C upon receipt. The kit should be equilibrated to room temperature (20-23°C) before assay.
2. Store **1x** Assay Buffer at 4°C.
3. Remove any unneeded strips from Human Soluble ST2 antibody-Coated plate, reseal them in zip-lock foil and keep at 4°C.
4. Keep rehydrated solution of Human Soluble ST2 Standard, Biotinylated anti-Human Soluble ST2 Detection Antibody and SA-HRP at 4°C. Prepare only the required amount.

NOTE:

1. It is recommended that the solutions be used on the same day of rehydration.
3. After adding Stop Solution, read the plate within 20 minutes.

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