

# Quantikine<sup>®</sup> ELISA

## Human ST2/IL-1 R4 Immunoassay

Catalog Number DST200

For the quantitative determination of human Suppression of Tumorigenicity 2 (ST2) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.  
For research use only. Not for use in diagnostic procedures.

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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). ST2 is expressed on the surface of mast cells, activated Th2 cells, macrophages, and cardiac myocytes (3-8). 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 Toll/Interleukin-1 Receptor (TIR) domain (9, 10). 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 (10). Within the ECD, human ST2 shares 68% and 64% aa sequence identity with mouse and rat ST2, respectively.

ST2 binds IL-33, a pro-inflammatory IL-1 family cytokine with intracellular and extracellular activities. IL-33 is constitutively expressed in smooth muscle and airway epithelia (3). It is upregulated by inflammatory stimulation in these cells, keratinocytes, and dermal fibroblasts and by mechanical strain in cardiac fibroblasts (3, 11). Like IL-1, the N-terminal propeptide of IL-33 is cleaved intracellularly to release the C-terminal fragment which is exported as the active cytokine (3, 12). IL-33 binding induces the association of transmembrane ST2 with IL-1 R AcP, a shared signaling subunit that also associates with IL-1 RI and IL-1 Rrp2/IL-1 R6 (13, 14). Soluble ST2 also binds IL-33 and functions as a decoy receptor that blocks the ability of IL-33 to signal through transmembrane ST2 (11, 13, 15-17).

Secreted IL-33 promotes Th2-biased immune responses, resulting in eosinophilia and allergic inflammation (18). It induces the upregulation of inflammatory cytokines and chemokines in Th2 cells and mast cells (3, 19, 20). It also functions as a chemoattractant for Th2 cells to sites of inflammation (21).

In addition to its role in promoting mast cell and Th2 dependent inflammation, transmembrane ST2 activation enhances inflammation-associated hypernociception and protects from atherosclerosis and cardiac myocyte hypertrophy (11, 15, 16). The soluble ST2 isoform is elevated in the serum under inflammatory conditions including allergic asthma, sepsis, trauma, dengue fever, and pulmonary disease (17, 22-25). Serum ST2 elevation is also associated with multiple aspects of heart failure including aortic stenosis, congestive cardiomyopathy, and risk of cardiovascular heart failure and death (26-31).

The Quantikine Human ST2/IL-1 R4 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human ST2 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human ST2 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ST2 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human ST2.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ST2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ST2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ST2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ST2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human ST2 Microplate	893762	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ST2.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ST2 Standard	893764	2 vials of recombinant human ST2 in a buffered protein solution with preservatives; lyophilized. <i>Refer to vial label for reconstitution volume.</i>	Discard after use. Use a fresh standard for each assay.
Human ST2 Conjugate	893763	12 mL of polyclonal antibody specific for human ST2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-63	895352	12 mL of a buffered protein solution with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a buffered protein base with preservatives. <i>Use diluted 1:4 in this assay.</i>	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of a diluted hydrochloric acid solution.	
Plate Sealers	N/A	4 adhesive strips.	

\* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards and samples.
- Human ST2 Controls (optional; R&D Systems, Catalog # QC162).

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay.*

## SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 10  $\mu$ L of sample + 190  $\mu$ L of Calibrator Diluent RD5-26 (diluted 1:4)\*.

\*See Reagent Preparation section.

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## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

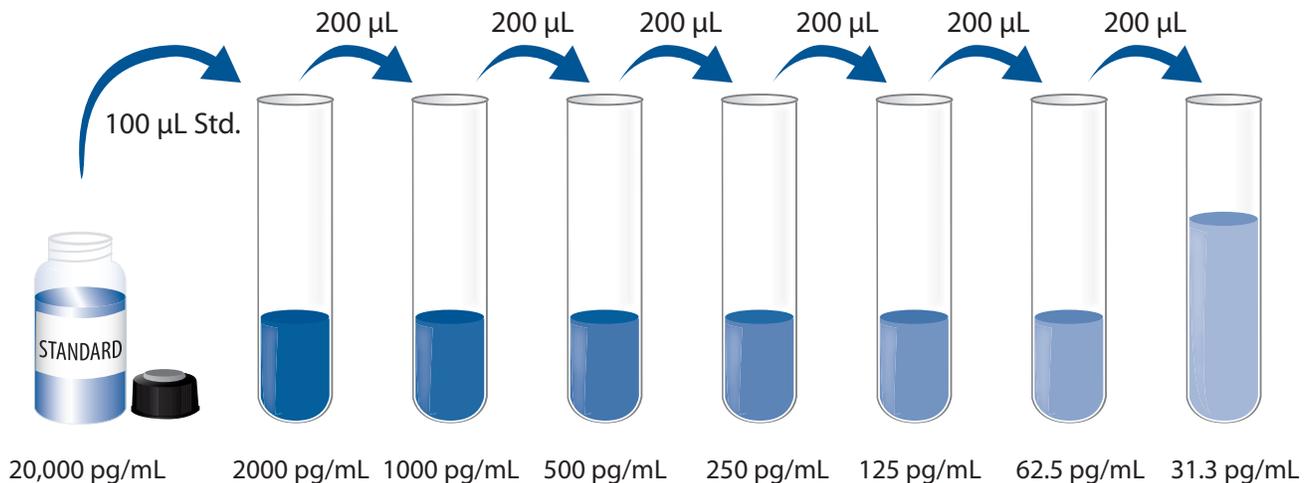
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100  $\mu$ L of the resultant mixture is required per well.

**Calibrator Diluent RD5-26 (diluted 1:4)** - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

**Human ST2 Standard - Refer to the vial label for reconstitution volume.** Reconstitute the Human ST2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900  $\mu$ L of Calibrator Diluent RD5-26 (diluted 1:4) into the 2000 pg/mL tube. Pipette 200  $\mu$ L Calibrator Diluent RD5-26 (diluted 1:4) into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50  $\mu\text{L}$  of Assay Diluent RD1-63 to each well.
4. Add 50  $\mu\text{L}$  of Standard, control, or sample\* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on the benchtop.
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 (400  $\mu\text{L}$ ) 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  $\mu\text{L}$  of Human ST2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the benchtop.
7. Repeat the aspiration/wash as in step 5.
8. Add 100  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 100  $\mu\text{L}$  of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

\*Samples may require dilution. See Sample Preparation section.

## CALCULATION OF RESULTS

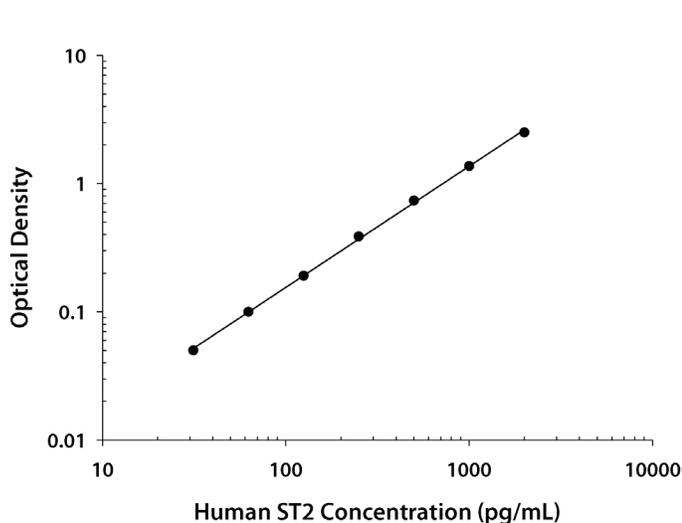
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 a log/log graph. The data may be linearized by plotting the log of the human ST2 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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

## TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.040	0.038	—
31.3	0.085 0.090	0.088	0.050
62.5	0.137 0.138	0.138	0.100
125	0.228 0.229	0.229	0.191
250	0.420 0.430	0.425	0.387
500	0.764 0.783	0.774	0.736
1000	1.373 1.442	1.408	1.370
2000	2.536 2.552	2.544	2.506

## PRECISION

### Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

### Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	273	628	1027	262	642	1064
Standard deviation	15.2	27.5	46.3	18.7	34.7	67.2
CV (%)	5.6	4.4	4.5	7.1	5.4	6.3

## RECOVERY

The recovery of human ST2 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	89-106%

## LINEARITY

To assess the linearity of the assay, samples containing or spiked with high concentrations of human ST2 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	105	105	104	105
	Range (%)	102-109	97-109	98-109	98-110
1:4	Average % of Expected	105	104	103	107
	Range (%)	102-112	98-109	96-110	98-115
1:8	Average % of Expected	102	102	104	104
	Range (%)	90-111	91-113	93-110	92-112
1:16	Average % of Expected	98	100	100	98
	Range (%)	85-113	87-112	89-111	89-112

\*Samples were diluted prior to assay as described in the Sample Preparation section.

## SENSITIVITY

Forty-five assays were evaluated and the minimum detectable dose (MDD) of human ST2 ranged from 2.45-13.5 pg/mL. The mean MDD was 5.1 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human ST2 produced at R&D Systems.

## SAMPLE VALUES

**Serum/Plasma** - Samples from apparently healthy volunteers were evaluated for the presence of human ST2 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	13.0	6.74-20.4	4.15
Heparin plasma (n=35)	12.3	5.72-19.8	4.10
EDTA plasma (n=35)	12.2	4.90-19.9	4.08

### Cell Culture Supernates:

BJAB human Burkitt's lymphoma cells ( $1 \times 10^5$  cells/mL) were seeded in DMEM supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 126 pg/mL.

BUD-8 human fibroblast cells were seeded in MEM NEAA 90% supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 273 pg/mL.

U-87 MG human glioblastoma/astrocytoma cells ( $2.1 \times 10^5$  cells/mL) were seeded in MEM supplemented with 10% fetal calf serum, 1% sodium pyruvate, and 2 mM L-glutamine until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 63.9 pg/mL.

HUVEC human umbilical vein endothelial cells ( $1 \times 10^4$  cells/mL) were seeded in EGM-2 media until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 29,920 pg/mL.

HUT-78 human cutaneous T cell lymphoma cells ( $1 \times 10^4$  cells/mL) were seeded in RPMI supplemented with 10% fetal calf serum, 5  $\mu$ M  $\beta$ -mercaptoethanol, and 10 ng/mL recombinant human IL-2 until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 54.9 pg/mL.

## SPECIFICITY

This assay recognizes natural and recombinant human ST2. This assay also recognizes free ST2 and IL-33 complexed ST2.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human ST2 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

IL-1 $\alpha$	IL-1F10	IL-18
IL-1 $\beta$	IL-1ra	IL-18 R $\beta$
Pro-IL-1 $\beta$	IL-1 RI	IL-33
IL-1F5	IL-1 RII	Pro-IL-33
IL-1F6	IL-1 R AcP	Integrin $\alpha$ M/CD11b
IL-1F7	IL-1 RAPL1	SIGIRR
IL-1F8	IL-1 RAPL2	Siglec-2/CD22
IL-1F9	IL-1 Rrp2	

### Recombinant mouse:

IL-33
Pro-IL-33
ST2

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