ARBOR ASSAYS™
Interactive Assay Solutions™

DetectX®

Human ST2
Enzyme Immunoassay Kit

Catalog Number K055-H1

Sample Types Tested:

Serum, EDTA Plasma and Tissue Culture Media

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.

www.ArborAssays.com

WEB K055-H 240308

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BACKGROUND

ST2 (also known as growth STimulation expressed gene 2, IL1RL1, DER4, T1 and FIT-1) is a member of the Toll-like/IL-1-receptor superfamily. Members of this superfamily are defined by a common intracellular domain, the Toll/Interleukin-1 receptor (TIR) domain. This domain of ~160 amino acids is composed of a central five-stranded ß-sheet surrounded by five a-helices located on the cytosolic end of the protein. The interleukin-1 (IL-1) receptor family has several members, including the classical interleukin-1 receptor (IL-1R) and the interleukin-18 receptor (IL-18R). In 1989, one member of the family, ST2, was identified as an orphan receptor. Investigation into the function of ST2 revealed its participation in inflammatory processes, particularly regarding mast cells, type 2 CD4+ T-helper cells and the production of Th2-associated cytokines. ST2 was characterized as a specific cellular marker that differentiated Th2 from Th1 T-cells.

The gene for ST2 spans ~40 kb on human chromosome 2q12, and is part of the larger human IL-1 gene cluster of ~200 kb. ST2 is conserved across species, with homologues in the genomes of mouse (Mus musculus chromosome 1), rat (Rattus norvegicus chromosome 9) and fruitfly (homologues to the Drosophila melanogaster Toll protein). The ~37 kD unglycosylated secreted protein is converted into a 60–70 kD glycosylated product, which is the soluble form of ST2, sST2.

Clinical and experimental observations led to the association of ST2 with diseases such as asthma, pulmonary fibrosis, rheumatoid arthritis, collagen vascular diseases and septic shock. Serum levels of ST2 are elevated in patients with acute cases of bronchial asthma, and in emergency-room patients presenting with shortness of breath. Serum levels of ST2 can discriminate between heart failure and non-cardiovascular etiologies.

ASSAY PRINCIPLE

The DetectX® human ST2 EIA kit is designed to quantitatively measure ST2 present in a variety samples and tissue culture media. Please read the complete kit insert before performing this assay. A recombinant human ST2 standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with a monoclonal antibody to capture ST2 present in the sample. After a 60 minute incubation, the plate is washed. A biotinylated ST2 antibody is added and the plate incubated for an additional 60 minutes. Following a second wash, peroxidase conjugated streptavidin is added and the plate is incubated for 30 minutes and washed. Substrate is then added to the plate, which reacts with the bound peroxidase conjugated streptavidin. After an incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of the ST2 in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

RELATED PRODUCTS

DetectX® Kits	Catalog No.
Insulin ELISA Kit	K046-H1
Atrial Natriuretic Peptide (ANP) ELISA Kit	K026-H1/H5
Thyroxine (T_4) ELISA Kit	K050-H1/H5
Cortisol ELISA Kits	K003-H1/H5
Glucose Colorimetric Detection Kit (2 Plate)	K039-H1
Glucose Fluorescent Detection Kit (2 Plate)	K039-F1

SUPPLIED COMPONENTS

Clear Coated 96 Well Plate
Clear plastic microplate with break-apart strips coated with mouse anti-human ST2.
One Plate Catalog Number C205-1EA

Human ST2 Standard
1 ng of recombinant human ST2 lyophilized stored in a ziplock pouch with desiccant.
2 Each Catalog Number C204-1EA

DetectX® ST2 Detection Antibody
Biotinylated antibody to human ST2.
5 mL Catalog Number C202-5ML

Streptavidin-Peroxidase Conjugate
Streptavidin-HRP in a special stabilizing solution.
5 mL Catalog Number C203-5ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
28 mL Catalog Number X065-28ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL Catalog Number X007-30ML

TMB Substrate
11 mL Catalog Number X019-11ML

Stop Solution
A 1M hydrochloric acid solution. **CAUSTIC**.
5 mL Catalog Number X020-5ML

Plate Sealer
3 each Catalog Number X002-1EA

STORAGE INSTRUCTIONS

All components of this kit should be stored at 4°C until the expiration date of the kit.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Protease inhibitors must be added to Assay Buffer. We recommend:

Phenylmethane sulfonyl fluoride (PMSF), such as Sigma 78830 at 100 mM in ethanol.

A universal protease inhibitor cocktail (PIC), such as Sigma P1860 or Roche cOmplete ULTRA Tablets, 058929700001.

Repeater pipet with disposable tips capable of dispensing 25, 50, and 100 µL.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for human serum, plasma, and tissue culture media (TCM) samples only. Samples containing visible particulate should be centrifuged prior to using. This assay has low or no reactivity to rat or mouse ST2. The end user should test this kit for application in their samples.

SAMPLE PREPARATION

Serum and EDTA Plasma Samples

Serum and plasma samples must be diluted ≥ 1:4 in Assay Buffer.

Tissue Culture Media Samples

TCM samples should be diluted in TCM and read off the standard curve generated in the same TCM.

Any samples with concentrations outside the standard curve range should be diluted further with Assay Buffer, as appropriate, to obtain readings within the standard curve range.

Use all samples within 2 hours of dilution.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes.

Assay Buffer

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable for 3 months at 4°C.

Prior to Running Assay

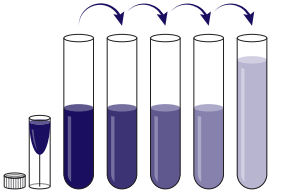
Add 0.5 µL of PIC to each mL of diluted Assay Buffer. 1 mM PMSF must be added to the diluted Assay Buffer. **Use within 8 hours.**

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

Standard Preparation

Allow the ziplock bag to warm to room temperature prior to opening. Remove the vial of standard and add 500 µL of Assay Buffer to the vial of ST2 standard to generate the 2,000 pg/mL Standard 1. Allow to sit at room temperature for 5 minutes. Vortex the vial. Label test tubes as #2 through #7. Pipet 200 µL of Assay Buffer into tubes #2 to #7. Carefully add 200 µL of Standard 1 to tube #2 and vortex completely. Take 200 µL of the ST2 solution in tube #2 and add it to tube #3 and vortex completely. Repeat the serial dilutions for tubes #4 through #7. The concentration of ST2 in the tubes #1 through #7 will be 2,000, 1,000, 500, 250, 125, 62.5 and 31.25 pg/mL.



Use all Standards within 2 hour of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	**500**	200	200	200	200	200	200
Addition	Vial	Std 1	Std 2	Std 3	Std 4	Std 5	Std 5
Volume of Addition (µL)	**0**	200	200	200	200	200	200
Final Conc (pg/mL)	2,000	1,000	500	250	125	62.5	31.25

ASSAY PROTOCOL

Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine ST2 concentrations.

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification.
2. Determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
3. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of Assay Buffer into the zero standard wells.
4. Cover the plate with the plate sealer and shake at room temperautre for **1 hour**. We recommend shaking at around 700–900 rpm.
5. Aspirate the plate and wash each well 4 times with 300 µL of diluted Wash Buffer.
6. Add 50 µL of the DetectX® ST2 Antibody to each well using a repeater pipet.
7. Cover the plate with the plate sealer and shake at room temperautre for **1 hour.** We recommend shaking at around 700–900 rpm.
8. Aspirate the plate and wash each well 4 times with 300 µL of diluted Wash Buffer.
9. Add 50 µL of the Streptavidin Peroxidase Conjugate to each well using a repeater pipet.
10. Cover the plate with the plate sealer and shake at room temperautre for **30 minutes**. We recommend shaking at around 700–900 rpm.
11. Aspirate the plate and wash each well 4 times with 300 µL of diluted Wash Buffer.
12. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
13. Incubate the plate at room temperature for 30 minutes without shaking.
14. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
15. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
16. Use the plate reader's built-in 4PLC software capabilities to calculate ST2 concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

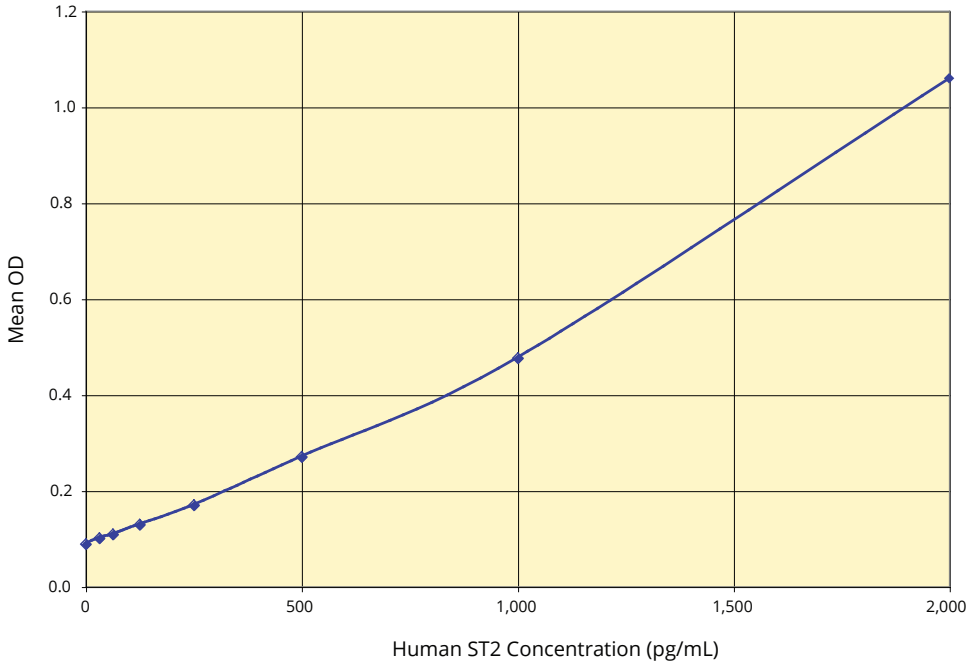
Or use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-human-st2-enzyme-immunoassay-kit-k055.assay

TYPICAL DATA

Sample	Mean OD	ST2 Conc. (pg/mL)
Standard 1	1.060	2,000
Standard 2	0.477	1000
Standard 3	0.271	500
Standard 4	0.170	250
Standard 5	0.130	125
Standard 6	0.110	62.5
Standard 7	0.102	31.25
Zero	0.089	0
Sample 1	0.488	994.6
Sample 2	0.265	514.3

Always run your own standard curve for calculation of results. Do not use this data.

Typical Standard Curves



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

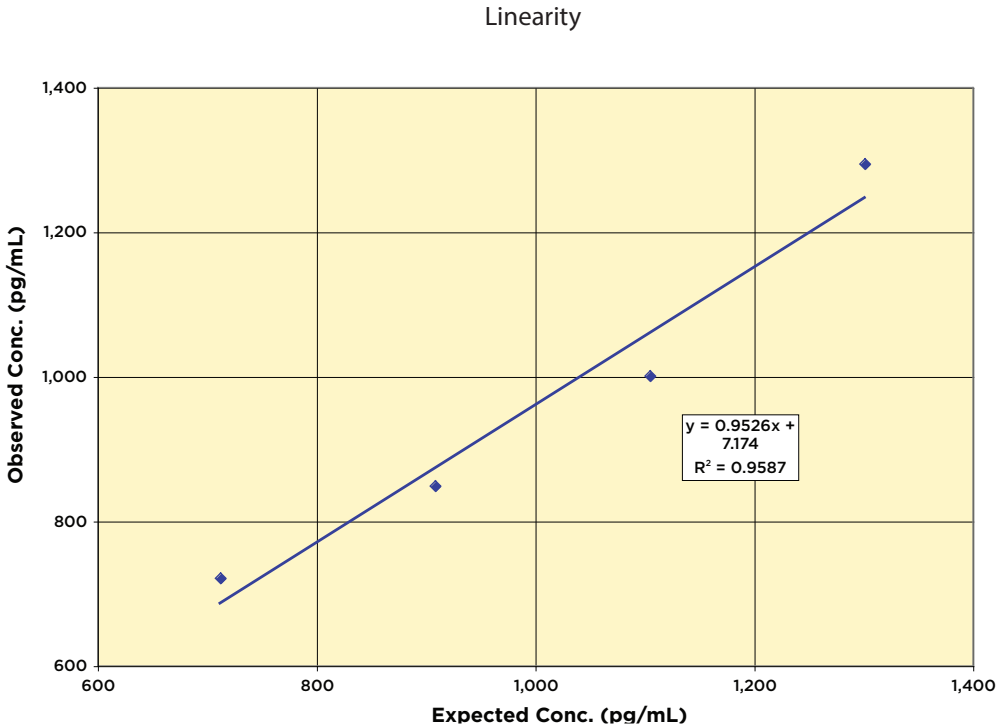
Sensitivity was calculated by comparing the OD's for eighteen wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero standard along the standard curve. **Sensitivity was determined as 30.5 pg/mL.**

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample. **Limit of Detection was determined as 13.8 pg/mL.**

Linearity

Linearity was determined by taking two samples, one with a low ST2 level of 516.1 pg/mL and one with a higher diluted level of 1,497.8 pg/mL and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

High Serum	Low Serum	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
80%	20%	1,301.4	1,294.1	99.4
60%	40%	1,105.1	1,000.9	90.6
40%	60%	908.7	848.9	93.4
20%	80%	712.4	721.35	101.3%
			Mean Recovery	**96.2%**



Intra Assay Precision

Three samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated ST2 concentrations were:

Sample	ST2 Conc. (pg/mL)	%CV
1	1,648.7	2.2
2	1,136.5	2.6
3	585.3	8.2

Inter Assay Precision

Three samples were diluted with Assay Buffer and run in duplicates in sixteen assays run over multiple days by three operators. The mean and precision of the calculated ST2 concentrations were:

Sample	ST2 Conc. (pg/mL)	%CV
1	1,463.7	7.5
2	990.0	8.1
3	489.3	9.8

SAMPLE VALUES

Normal, and disease human plasma samples from patients with heart disease and diabetes, were tested in the assay. Neat sample values ranged from over 350 to over 1,040 pg/mL with an average of 589.1 pg/mL. Eighteen human serum samples from normal and disease patients were tested in the assay. Neat sample values ranged from 462.8 to 1,045 pg/mL.

CROSS REACTIVITY

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Steroid	Cross Reactivity (%)
Human ST2	100%
Mouse ST2/IL-1 R4/ Fc Chimera	0.30%
IL-1a	<0.1%
IL-1ß	<0.1%
IL-1 RI	<0.1%
IL-1 RII	<0.1%
IL-1ra	<0.1%
IL-1 RAcP/Fc Chimera	<0.1%

LIMITED WARRANTY

Arbor Assays warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

For details concerning this kit or to order any of our products please contact us:

Arbor Assays

1514 Eisenhower Place
Ann Arbor, Michigan 48108 USA

Phone: 734-677-1774

Fax: 734-677-6860

Web: www.ArborAssays.com

Email Addresses:

Info@ArborAssays.com

Orders@ArborAssays.com

Technical@ArborAssays.com

Contracts@ArborAssays.com

OFFICIAL SUPPLIER TO ISWE

Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with EIA kits for wildlife conservation research.

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