Datasheet for ABIN2866594

IL1RL1 ELISA Kit

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Overview

Quantity:	96 tests
Target:	IL1RL1
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	The DetectX® human ST2 EIA kit is designed to quantitatively measure ST2 present in a variety samples and tissue culture media.
Brand:	DetectX®
Sample Type:	Serum, Plasma (EDTA), Tissue Culture Medium
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Clear Coated 96 Well Plate Clear plastic microplate with break-apart strips coated with mouse anti-human ST2. One Plate Human ST2 Standard 1 ng of recombinant human ST2 lyophilized stored in a ziplock pouch with desiccant. 2 each DetectX® ST2 Detection Antibody Biotinylated antibody to human ST 2. 5 mL Streptavidin-Peroxidase Conjugate Streptavidin-HRP in a special stabilizing solution. 5 mL Assay buffer Concentrate A 5X concentrate that should be diluted with deionized or distilled water. 28 mL Wash buffer Concentrate A 20X concentrate that should be diluted with deionized or distilled

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	water. 30 mL TMB Substrate 11 mL Stop Solution A 1N hydrochloric acid solution. Caustic. 5 mL Plate Sealer 3 each
Material not included:	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 µL, 50 µL 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.

Target Details

Target:	IL1RL1
Alternative Name:	IL1RL1 (IL1RL1 Products)
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 cy- tosolic 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 (homo- logues 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

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	association of ST2 with diseases such as asthma, pulmonary fibrosis, rheumatoid arthritis, collagen vascular diseases and septic shock. Serum lev- els 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.

Application Details

Application Notes:	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.
Plate:	Pre-coated
Protocol:	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 con- jugated 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.
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 Procedure:	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 4ºC. 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. 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. 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. 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.

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	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.
Restrictions:	For Research Use only

Handling

Precaution of Use:	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. The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.
Storage:	4 °C
Storage Comment:	All components of this kit should be stored at 4°C until the expiration date of the kit.

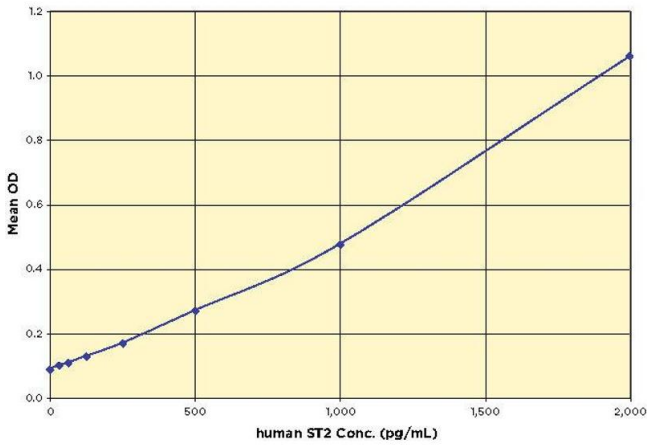
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Mean OD
0
500
1,000
1,500
2,000
human ST2 Conc. (pg/mL)

Image 1.