Human ST2 Sandwich ELISA Kit Datasheet

For the quantitative detection of human ST2 concentrations in serum, plasma and cell culture supernatants.

General Information

Catalogue Number	KE00055
Product Name	Human ST2 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	31.25-2000 pg/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	9173
SwissProt	Q01638

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 2000 pg/bottle; lyophilized*	2 bottles	
Detection Antibody, biotinylated (100X) - 120 μL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 μL/vial	1 vial	
Sample Diluent PT 3-af - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

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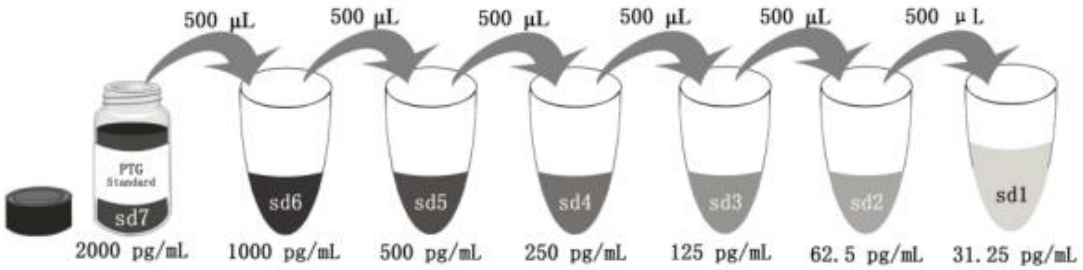
NB: Do not use the kit after the expiration date.
Sample Diluent PT 3-af is for protein standard and samples.
Detection Diluent is for Detection antibody and Streptavidin-HRP.
*Add **1 mL** Sample Diluent PT 3-af in protein standard. This reconstitution gives a stock solution of 2000 pg/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 3-af	**1000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00055 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The ST2 ELISA kit is to be used to detect and quantify protein levels of endogenous ST2. The assay recognizes human ST2. An antibody specific for ST2 has been pre-coated onto the microwells. The ST2 protein in samples is captured by the coated antibody after incubation. Following extensive washing, another antibody of biotinylated specific for ST2 is added to detect the captured ST2 protein. For signal development, Sterptravidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

Protein ST2, also known as IL1RL1, is a member of the interleukin 1 receptor family. The gene of ST2 encodes three splice variants: a soluble secreted form (sST2), a transmembrane receptor form (ST2L), and a variant form of ST2 (ST2V). Interleukin-33 (IL-33) has been identified as a functional ligand of ST2L. IL-33 exerts its cellular functions by binding a receptor complex composed of ST2L and IL-1R accessory protein (IL-1RAcP). The interaction of IL-33 and ST2L activates mitogen-activated protein kinases and several biochemical pathways. The end of these reactions is the activation of the inhibitor of nuclear factor-kB (NF-kB) kinase complex, triggering NF-kB activity. sST2 seems to act as a decoy-receptor for IL-33: it binds IL-33 thus subtracting such a molecule from the interaction with ST2L. sST2 is formed by many cells and sST2 level is increased as response to myocardial stress, as well as in inflammatory conditions, including allergic asthma.

Sample Preparation

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The serum or plasma samples may require proper dilution to fall within the range of the assay. The serum or plasma is better to be diluted 1:5 or 1:10 before assay, 1:8 or 1:16 dilution is recommended for cell culture supernatants.

Safety Notes

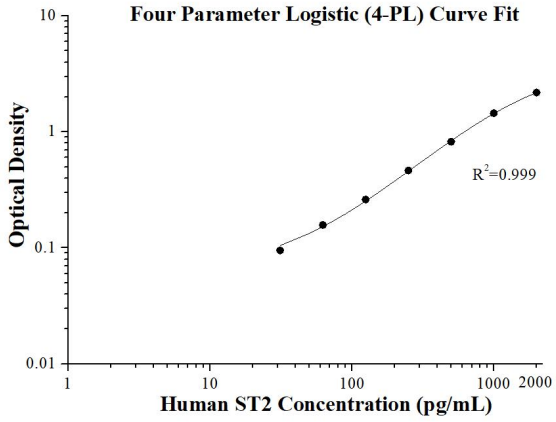
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.083 0.080	0.082	-
31.25	0.176 0.177	0.177	0.095
62.5	0.24 0.238	0.239	0.158
125	0.341 0.343	0.342	0.261
250	0.55 0.541	0.546	0.464
500	0.900 0.907	0.904	0.822
1000	1.524 1.537	1.531	1.449
2000	2.286 2.241	2.264	2.182

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Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	1,058.5	30.8	2.9
2	20	247.2	5.6	2.3
3	20	61.9	1.6	2.6

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	1,054.8	40.8	3.9
2	20	257.3	7.1	2.7
3	20	72.4	4.4	6.1

Recovery

The recovery of ST2 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human plasma	1:8	90	83-102
	1:16	91	82-103
Cell culture supernatants	1:32	97	80-123
	1:64	104	91-118

Sample Values

Sample Type	Mean of Detectable (**ng/mL**)	Range (**ng/mL**)
Human serum (n=21)	6.0	1.2-19.0

HUVEC human umbilical vein endothelial cells (1 x 10^6 cells/mL) were seeded in Endothelial Cell Medium(ECM) supplemented until confluent. An aliquot of the cell culture supernate was removed, assayed for human ST2, and measured 10,559 pg/mL.

Sensitivity

The minimum detectable dose of human ST2 is 1.8 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

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Linearity

To assess the linearity of the assay, samples were diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. ((The plasma samples were initially diluted 1:2,the cell culture supernatants were initially diluted 1:4)

		Human plasma	Cell culture supernatants
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	113	109
	Range (%)	105-122	99-119
1:8	Average% of Expected	116	108
	Range (%)	103-124	100-115
1:16	Average% of Expected	112	117
	Range (%)	100-124	114-120

References

1. Kuroiwa K, et al. Construction of ELISA system to quantify human ST2 protein in sera of patients. Hybridoma. 19(2):151-9 (2000).
2. Pecaric-Petkovic T, et al. Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. Blood. 113(7):1526-34 (2009).
3. Ciccone MM, et al. A novel cardiac bio-marker: ST2: a review. Molecules. 18(12):15314-28 (2013).
4. Pascual-Figal DA, et al. The biology of ST2: the International ST2 Consensus Panel. Am J Cardiol. 115(7 Suppl):3B-7B (2015).

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