Human sST2 ELISA

Cat. No.: BA1034

Enzyme Immunoassay for the quantitative determination sST2 in human serum and plasma.

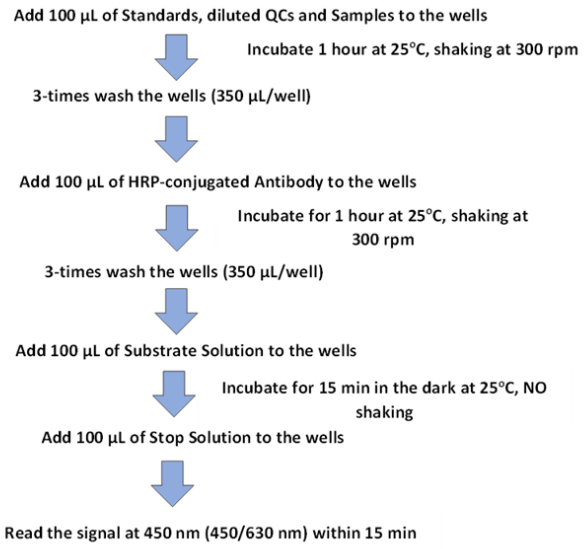
sST2 (Soluble suppression of tumorigenicity 2) is a member of the Interleukin-1 receptor family. Like other ligands in the IL-1 family, the binding of IL-33 and ST2L on the inflammatory cell membrane activates subsequent intracellular signalling and mediates its pro-inflammatory action. It is raised in various cardiovascular diseases. sST2 significantly predicts disease severity and mortality in cardiovascular disease and is a good predictor of mortality in patients with stable coronary artery disease and chronic heart failure.

PRINCIPLE OF sST2 ELISA

The microtiter plate is coated with the antibody specifically binding the sST2. The human serum or plasma is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with HRP-labelled detection antibody. Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of sST2 in the specimen. The concentration of sST2 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	***Qty.***
Antibody Coated Microtiter Plate	96 wells
Antibody-HRP Conjugate	13 mL
Master Standard (lyophilized)	2 vials
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ±2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ±2°C.

Preparation of Standards

Reconstitute lyophilized Human sST2 Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min. The concentration of human sST2 in Master Standard is 6 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 6 ng/mL (lyophilised)	See CofA	6 ng/ml
Std2	250 µL of Std1	250 µL	3 ng/mL
Std3	250 µL of Std2	250 µL	1.5 ng/mL
Std4	250 µL of Std3	250 µL	0.75 ng/mL
Std5	250 µL of Std4	250 µL	0.375 ng/mL
Std6	250 µL of Std5	250 µL	0.1875 ng/mL
Blank	-	250 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min and dilute them 1:20 in Dilution Buffer, prior to use, see Preparation of samples.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:20, i.e., 10 µL of sample + 190 µL of Dilution Buffer for singlets and 15 µL of sample + 285 µL of Dilution Buffer for duplicates.

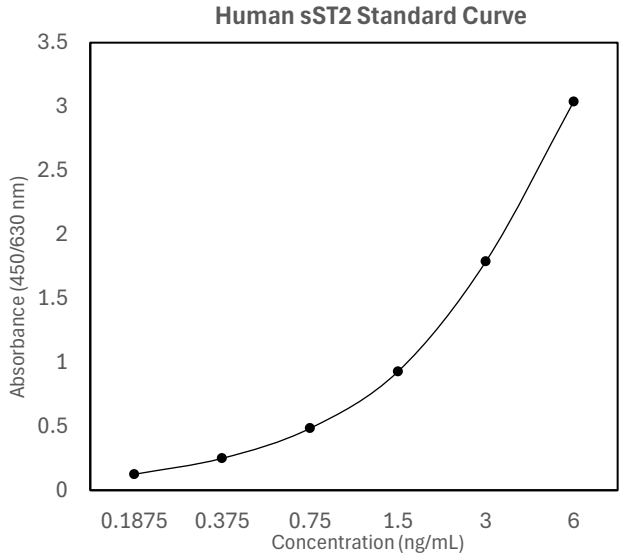
Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate **1 hour** at 25°C ±2°C, shaking at **350 rpm**.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of HRP-labelled Antibody Conjugate into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at **350 rpm**.
5. Wash the wells as described in point 3.
6. Pipette 100 µL Substrate solution, incubate for **15 min,** at 25°C ±2°C**.** Avoid exposure to the light during this step.
7. Pipette 100 µL of STOP solution.
8. Read the signal at 450 or 450/630 nm within 15 min.

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 5-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:20 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human sST2 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 20 pg/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	7.04	0.741	10.5
2	44.46	1.506	3.4

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	8.47	1.164	13.8
2	44.39	5.322	12.0

3. Accuracy

Dilution linearity

Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		50.74	-	-
	2x	24.82	25.37	98
	4x	12.32	12.68	97
	8x	6.13	6.34	97
2		56.14	-	-
	2x	26.44	28.07	94
	4x	13.81	14.03	98
	8x	6.53	7.02	93

Spiking Recovery

Sample	Spike (ng/mL)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	13.01	-	-
	30	46.39	43.01	108
	15	32.09	28.01	115
	7.5	23.38	20.01	114
2	-	7. 64	-	-
	30	42.84	37.64	114
	15	26.11	22.64	115
	7.5	17.58	15.14	116

POPULATION AND PRELIMINARY CLINICAL DATA

Normal value: median +/- SD 8.44 +/- 10.79 ng/ml,
Reference range - 95% of healthy individuals: 0 – 32.10 ng/ml
Risk value (HF development, fibrosis) > 35 ng/ml
High risk value (HF development, fibrosis) > 70 ng/ml

RESOURCES

[1] Ip C, Luk KS, Yuen VLC, Chiang L, Chan CK, Ho K, Gong M, Lee TTL, Leung KSK, Roever L, Bazoukis G, Lampropoulos K, Li KHC, Tse G, Liu T; International Health Informatics Study (IHIS) Network. Soluble suppression of tumorigenicity 2 (sST2) for predicting disease severity or mortality outcomes in cardiovascular diseases: A systematic review and *meta*-analysis. Int J Cardiol Heart Vasc. 2021 Oct 18;37:100887. doi: 10.1016/j.ijcha.2021.100887. PMID: 34712771; PMCID: PMC8528731.