ST2 – a marker of cardiac stress

ST2 is a member of the interleukin-1 receptor family and it is also known as interleukin-1 receptor-like 1 (IL1RL-1). ST2 exists in two isoforms: a transmembrane or cellular (ST2L) and soluble or circulating (sST2). ST2 is the receptor for interleukin-33 (IL-33), which is an IL-1-like cytokine that is secret-ed by living cells in response to cell damage. The IL-33 exerts its effects by binding to the transmembrane receptor ST2L isoform. The interaction of IL-33 and ST2L has been shown to be cardioprotective, reducing myocardial fibrosis, cardiomyocyte hypertrophy, apoptosis, and improving myocardial function. The IL-33/ST2 system is upregulated in cardiomyocytes and fibroblasts in response to cardiac injury. sST2 avidly binds to IL-33 and competes with ST2L. The interaction of the soluble ST2 with IL-33 blocks the IL-33/ST2L system and as a result eliminates the cardioprotective pathway of the IL-33/ST2L interaction.

ST2L contains an extracellular domain of three immunoglobulin-like motifs, a transmembrane segment, and an intracellular cytoplasmic domain, whereas sST2 lacks the transmembrane and cytoplasmic domains. Three linked immunoglobulin-like motifs of sST2 have a total length of 310 amino acid residues. The expression of sST2 is largely inducible and it is almost ubiquitous in living cells, such as resting fibroblasts. It has been suggested that sST2 is produced by both cardiac fibroblasts and cardiomyocytes in response to cardiac injury or cardiac stress, macrovascular (aortic and coronary artery), and heart microvascular endothelial cells in response to diastolic load.

Reference values of ST2

According to the main reference ST2 assay (the Presage® ST2 assay manufactured by Critical Diagnostics) median ST2 concentrations are 23.6 ng/ml in males, and 16.2 ng/ml in females respectively. The median ST2 concentration in normal subjects was established as 18.8 ng/ml.

Clinical value of ST2

A sST2 is a biomarker that is used for additive risk stratification and prognosis of patients with heart failure (HF). In contrast to BNP and NT-proBNP, ST2 is not affected by confounding factors such as age, body mass index, and impaired renal function. Unlike many other cardiac biomarkers, the levels of ST2 alter quickly in response to changes in the patient's condition. This means that it helps physicians to take an appropriate course of action faster. Elevated levels of sST2 in acute as well as chronic HF patients (>35 ng/ml) are strongly associated with the measures of HF severity and they predict both recurrent hospitalization and mortality.

Measured levels of ST2 in chronic HF patients can be used for therapy evaluation and accordingly, decreased levels of sST2 that are responsive to medical treatment are associated with better outcomes for patients. According to most publications, the ST2 is an independent predictor of all-cause cardiac mortality and it provides complementary prognostic information not only for NT-proBNP (or BNP), but also for high-sensitivity cardiac troponin T (hs-cTnT) assays.

Reagents for the ST2 immunoassay development

Hytest provides several monoclonal antibodies (MAbs) that are specific to sST2 (Cat.# 4ST2). In addition, we offer recombinant ST2 antigen (Cat.# 8STR4) that can be used as a standard or calibrator in immunoassays.

CLINICAL UTILITY

- **Prognostic marker of heart failure**

MONOCLONAL ANTIBODIES SPECIFIC TO ST2

Our MAbs that are specific to ST2 are well-characterized and they may be used for the development of sensitive and precise immunoassays. These antibodies were obtained after the immunization of various animal species (mice, rabbits, and rats) with recombinant human ST2, expressed in mammalian cell line. The ST2 antibodies developed by Hytest specialists are both *in vitro* produced hybridoma MAbs, as well as recombinant and recombinant chimeric MAbs (see the ordering information).

Different types of antibodies can work differently in various applications. Therefore, we give our customers the opportunity to choose the most suitable antibodies for their platforms. Hytest MAbs that are specific to ST2 were studied in different immunoassay formats, such as sandwich ELISA and immunofluorescent (FIA) assays. Both assay platforms can be used for the development of high-sensitive and precise ST2-specific immunoassays.

Sandwich immunoassays

For the detection of ST2 in plasma samples of HF patients we recommend different MAb combinations (see Table 1). Some of them have better characteristics (sensitivity) when used in the sandwich ELISA platform, whereas others have better characteristics (assay time) when used in the sandwich FIA platform.

All recommended capture-detection pairs that were tested in the sandwich ELISA platform have sensitivity that is comparable with the sensitivity of the reference Presage® ST2 assay or better, while using serial dilutions of pooled plasma from HF patients. A calibration curve for the prototype assay S215-S103 is shown in Figure 1.

Table 1.

Recommended capture-detection pairs (prototype assays in sandwich ELISA format are marked in black, prototype assays in sandwich FIA format are marked in blue). Limit of detection (LoD) was determined as the mean of the blank (TBST buffer (ELISA)/assay buffer (FIA)) +2*SD. Recombinant ST2 (Cat.# 8STR4) reconstituted in a corresponding buffer was used as an analyte.

Capture MAb	Detection MAb	LoD (pg/ml) for ELISA/FIA	Assay format, ELISA/FIA
S207	S103	30	+/-
S207	S501	40	+/-
S501	S103	40/70	+/+
S215	S103	30/70	+/+ (in revers orientation)
S512	S103	40	+/-
S985	S501	90	-/+
S985	S512	70	-/+
S985	S103	50	-/+
S101	S985	70	-/+

The saturation rate was assessed for all the sandwich FIA assay prototypes. Based on the results, for assay prototypes using MAb S985 as capture and any of the MAbs S501, S512, or S103 as detection, as well as the assay prototype S101-S985, the saturation rate was higher compared to other MAb combinations. Therefore, these pairs can be recommended for rapid assay development.

Detection of ST2 in clinical samples

In order to conduct correlation studies between Hytest prototype immunoassays and the reference Presage® ST2 assay, the concentrations of ST2 were measured in the EDTA-plasma samples of 5 healthy subjects and 26 heart failure subjects respectively. Figure 2 shows the correlation studies between immunoassays that utilize MAbs S215-S103 and a commercially available diagnostic Presage® ST2 assay. The concentrations of ST2 obtained with these assays are directly comparable with the Pearson correlation coefficient of 0.98.

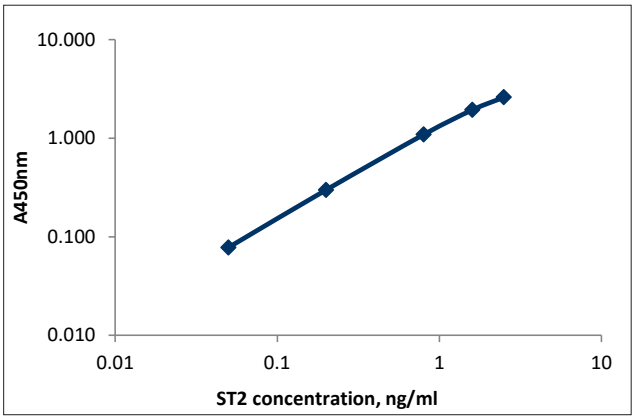

Figure 1.

Representative calibration curve for the ST2 prototype assay (S215-S103, sandwich ELISA), using recombinant ST2 (Hytest, Cat.# 8STR4) as the antigen.

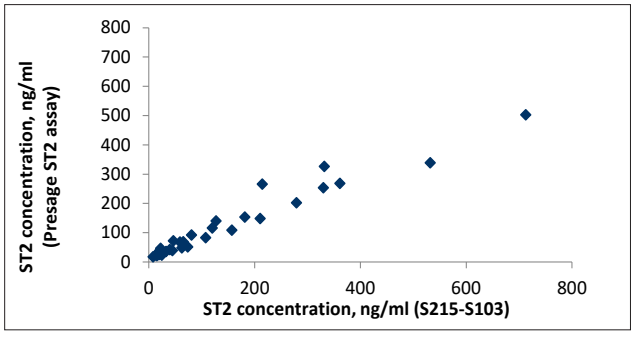

Figure 2.

ST2 concentrations that were obtained with the S215-S103 prototype immunoassay and the Presage® ST2 assay. The correlation coefficient (Pearson) between the assay S215 (capture) - S103 (detection) and the Presage ST2 assay is 0.98. Both assays are sandwich ELISA immunoassays and the protocol used was similar to that which was used in the Presage® ST2 assay.

RECOMBINANT ST2

Hytest recombinant ST2 (Cat.# 8STR4) is suitable for use as a standard or calibrator in immunoassays. Synthetic DNA fragment encoding amino acids 19-328 of human ST2 (accession number UniProtKB Q01638, ILRL1_HUMAN, isoform B) is used for recombinant protein expression in mammalian cell line. The recombinant ST2 consists of 310 amino acid residues and contains N-terminal His6-tag. The protein has a theoretical molecular weight (Mw) of approximately 36 kDa and it has several sites of N-glycosylation. SDS-PAGE of recombinant ST2 reveals that it migrates as a diffuse band because of N-glycosylation with apparent Mw of 58 kDa (see Figure 3).

The recombinant ST2 has a tendency to form dimers and it is therefore likely that the purified protein preparation contains a small amount of dimeric ST2 as well.

Recombinant ST2 tolerates freeze-thaw cycles

We tested the immunochemical stability of reconstituted recombinant ST2 after several repeated freeze-thaw cycles. The immunoreactivity, as measured using the S207-S103 prototype assay with the Europium label, did not change significantly when the protein was subjected to five freeze-thaw cycles (see Figure 4).

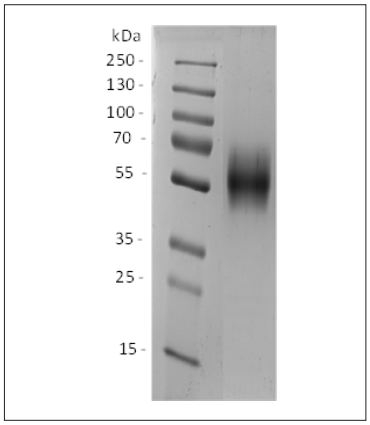

Figure 3.
SDS-PAGE of recombinant ST2 (Cat.# 8STR4) fragment under reducing conditions in a gradient gel (8-16%). 2 µg of purified protein was loaded on the gel. The purity of recombinant ST2 is more than 95%.

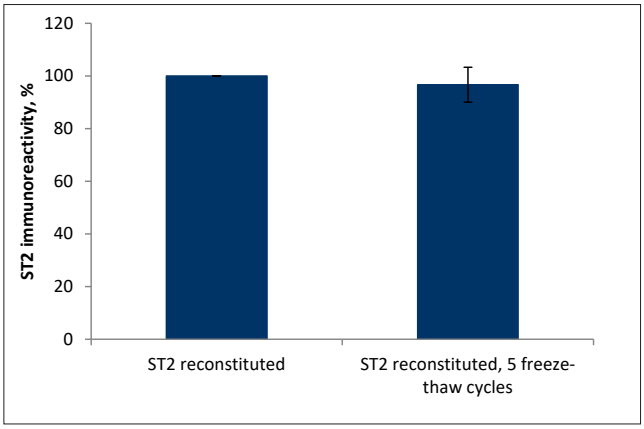

Figure 4.
Reconstituted recombinant ST2 subjected to 5 freeze-thaw cycles. Data are presented as a mean value of three tested dilutions of the sample with the standard deviation as a spread.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
ST2	4ST2	S985	IgG1	*In vitro*, FIA
		S101	IgG1	*In vitro*, FIA
		S103	IgG1	EIA, FIA, recombinant chimeric antibody
		S207	IgG	EIA, recombinant rabbit antibody
		S215	IgG	EIA, FIA, recombinant rabbit antibody
		S501	IgG1	EIA, FIA, recombinant chimeric antibody
		S512	IgG1	EIA, FIA, recombinant chimeric antibody

ANTIGEN

Product name	Cat. #	Purity	Source
ST2/ IL1RL1 protein, human, recombinant	8STR4	>95%	Recombinant

HYTEST LTD
Intelligate 1, 6th floor, Joukahaisenkatu 6 • FI-20520 Turku, FINLAND
Tel. +358 2 512 0900 • E-mail: hytest@hytest.fi • HYTEST.FI