ELISA Kit for Measuring Human S100A13

CircuLex S100A13 ELISA Kit

Cat# CY-8057

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Intended Use

The MBL Research Product **CircuLex S100A13 ELISA Kit** is used for the quantitative measurement of human S100A13 in cell culture supernatant and other biological samples.

This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.

Storage

- Upon receipt store all components at 4°C.
- Don't expose reagents to excessive light.

Introduction

S100A13, a small acidic protein that molecular weighs about 10-11 kDa, belongs to the S100 calcium-binding protein family. These family members share a common S100 calcium-binding motif and are involved in several regulatory functions that include protein phosphorylation, some enzyme activities, the dynamics of cytoskeletal components, transcription factors, and Ca2+ homeostasis, and also cell proliferation and differentiation. An interesting feature of the S100 proteins is that they are expressed by epithelial cells and fibroblasts in a cell-specific way.

The cDNA of human and murine S100A13 was first identified by screening expressed sequence tag data bases (1). The human S100A13 was shown to neighbor S100A1 on chromosome 1q21 (2). Expression of S100A13 mRNA has so far been detected in skeletal muscle, heart, kidney, pancreas, ovary, spleen, and small intestine. S100A13 seems to function in exocytosis, since it is one of the targets of two antiallergic drugs, amlexanox and cromolyn (3), which inhibit degranulation of mast cells (4). Recently, association of S100A13 with the fibroblast growth factor 1 (FGF-1)/p40 synaptotagmin-1 (p40Syn-1) aggregate was shown, and amlexanox is able to repress this release (5). These findings suggest that S100A13 might be involved in the regulation of FGF-1 and p40Syn-1 release in response to heat shock (6). Another possibility might be that S100A13 is secreted together with the FGF-1/p40Syn-1 aggregate.

Principle of the Assay

The CircuLex S100A13 ELISA Kit employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human S100A13 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human S100A13 present. After washing away any unbound substances, an HRP conjugated antibody specific for human S100A13 is added to the wells. Following a wash to remove any unbound antibody HRP conjugate, the remaining conjugate is allowed to react with the substrate H_2O_2-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of human S100A13. A standard curve is constructed by plotting absorbance values versus human S100A13 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

The CircuLex S100A13 ELISA Kit is designed to measure the concentration of human S100A13 from human serum/plasma, cultured human macrophages, or conditioned medium.

Summary of Procedure

Add 100 µL of diluted samples to the wells
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of HRP conjugated anti-S100A13 antibody
↓ Incubate for 60 minutes at room temp.
Wash the wells
↓
Add 100 µL of Substrate Reagent
↓ Incubate for 10-20 minutes at room temp.
Add 100 µL of Stop Solution
↓
Measure absorbance at 450 nm

Materials Provided

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

Microplate: One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with anti-S100A13 antibody as a capture antibody.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing Tween®-20

Standard Reconstitution Buffer: One tube containing 0.9 mL of 1X buffer; use for standard reconstitution. Ready to use.

Dilution Buffer: One bottle containing 50 mL of 1X buffer; use for sample dilution. Ready to use.

Human S100A13 Standard: One vial containing 44.8 ng of lyophilized recombinant human S100A13

HRP conjugated Detection Antibody: One bottle containing 12 mL of HRP (horseradish peroxidase) conjugated anti-S100A13 antibody. Ready to use.

Substrate Reagent: One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

Stop Solution: One bottle containing 20 mL of 1 N H_2SO_4. Ready to use.

Materials Required but not Provided

- **Pipettors:** 2-20 µL, 20-200 µL and 200-1,000 µL precision pipettors with disposable tips.
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation.
- **Vortex mixer**
- **(Optional) Microplate washer:** Manual washing is possible but not preferable.
- **Plate reader** capable of measuring absorbance in 96-well plates at dual wavelengths of 450 nm/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **(Optional) Software package facilitating data generation and analysis**
- **500 or 1,000 mL graduated cylinder**.
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- **Although we suggest to conduct experiments as outlined below, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user.**
- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. Care should be taken to avoid direct contact with these reagents.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with Substrate Solution which contains hydrogen peroxide.
- Avoid contact with Stop Solution which contains Sulfuric Acid.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of rat origin, and these reagents. In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological samples may be contaminated with infectious agents. Do not ingest, expose to open wounds or breathe aerosols. Wear protective gloves and dispose of biological samples properly.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

Sample Collection and Storage

Cell culture supernatant: Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles.

Other biological samples: Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles.

Detailed Protocol

The MBL Research Product **CircuLex S100A13 ELISA Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the human S100A13 Standard within the kit should be included in each assay as a calibrator. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

Preparation of Working Solutions

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready-to-use, with the exception of **10X Wash Buffer** and **Human S100A13 Standard**.

1. Prepare a working solution of Wash Buffer by adding 100 mL of the **10X Wash Buffer** to 900 mL of deionized (distilled) water. Mix well. Store at 4°C for two weeks or -20°C for long-term storage.

2. Reconstitute **Human S100A13 Standard** with **0.7 mL** of **Standard Reconstitution Buffer**. The concentration of the human S100A13 in vial should be **64 ng/mL**, which is referred to as a **Master Standard** of human S100A13.

Prepare Standard Solutions as follows:

Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 16 ng/mL standard (Std.1) serves as the highest standard. The **Dilution Buffer** serves as the zero standard (Blank).

	Volume of Standard	Dilution Buffer	Concentration
Std.1	150 µL of Master Standard	450 µL	16 ng/mL
Std.2	300 µL of Std. 1 (16 ng/mL)	300 µL	8 ng/mL
Std.3	300 µL of Std. 2 (8 ng/mL)	300 µL	4 ng/mL
Std.4	300 µL of Std. 3 (4 ng/mL)	300 µL	2 ng/mL
Std.5	300 µL of Std. 4 (2 ng/mL)	300 µL	1 ng/mL
Std.6	300 µL of Std. 5 (1 ng/mL)	300 µL	0.5 ng/mL
Std.7	300 µL of Std. 6 (0.5 ng/mL)	300 µL	0.25 ng/mL
Blank	-	300 µL	0 ng/mL

Note: Do not use a Repeating pipette. Change tips for every dilution. Wet tip with Dilution Buffer before dispensing. Unused portions of Master Standards should be aliquoted and stored at below -70°C immediately. Avoid multiple freeze and thaw cycles.

Sample Preparation

Dilute samples with **Dilution Buffer**.

- Cell culture supernatants may not require dilution.
- Other biological samples require 10- and 100- and 200- fold dilution or appropriate dilution.

Assay Procedure

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.

2. Dilute samples appropriately with **Dilution Buffer**. (See "Sample Preparation" above.)

3. Pipette **100 µL** of **Standard Solutions (Std1-Std7, Blank)** and **diluted samples** in duplicates, into the appropriate wells.

4. Incubate the plate **at room temperature (ca. 25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

5. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

6. Add **100 µL** of **HRP conjugated Detection Antibody** into each well.

7. Incubate the plate **at room temperature (ca. 25°C) for 60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.

8. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.

9. Add **100 µL** of **Substrate Reagent**. Avoid exposing the microtiter plate to direct sunlight. Return Substrate Reagent to 4°C immediately after the necessary volume is removed.

10. Incubate the plate **at room temperature (ca. 25°C) for 10-20 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker. The incubation time may be extended up to 30 minutes if the reaction temperature is below than 20°C.

11. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.

12. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

Note-1: Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
Note-2: Reliable standard curves are obtained when either O.D. values do not exceed 0.2 units for the blank (zero concentration), or 2.5 units for the highest standard concentration.
Note-3: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine human S100A13 concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculations

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the human S100A13 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human S100A13 concentration. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

1. The dose-response curve of this assay fits best to a sigmoidal 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 5-parameter logistic function. It is important to make an appropriate mathematical adjustment to accommodate for the dilution factor.

2. Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of calibrators versus log of the known concentration (X) of calibrators, using the four-parameter function. Alternatively, the logit log function can be used to linearize the calibration curve (i.e. logit of absorbance (Y) is plotted versus log of the known concentration (X) of calibrators).

Measurement Range

The measurement range is 0.25 ng/mL to 16 ng/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the human S100A13 concentration.

Troubleshooting

1. All samples and controls should be assayed in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.

2. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.

3. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the MBL Research Product **CircuLex S100A13 ELISA Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, kit reagents should be stored at 4°C, except the reconstituted human S100A13 Standard must be stored at below -70°C. Coated assay plates should be stored in the original foil bag sealed by the zip lock and containing a desiccant pack.

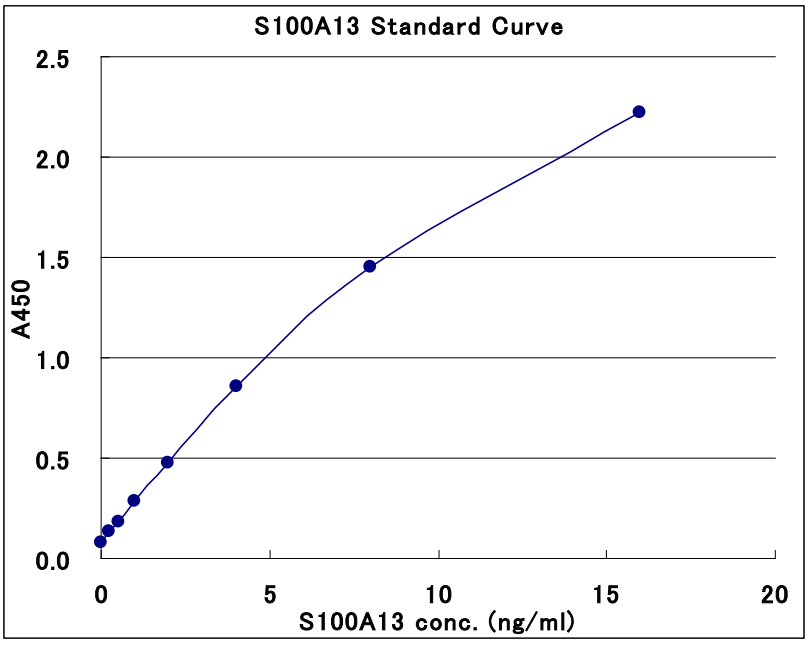
Assay Characteristics

1. Sensitivity

Twenty-four assays were evaluated and the minimum detectable dose (MDD) of human S100A13. The MDD (defined as such a concentration of human S100A13 giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 66.2 pg/ml of sample.

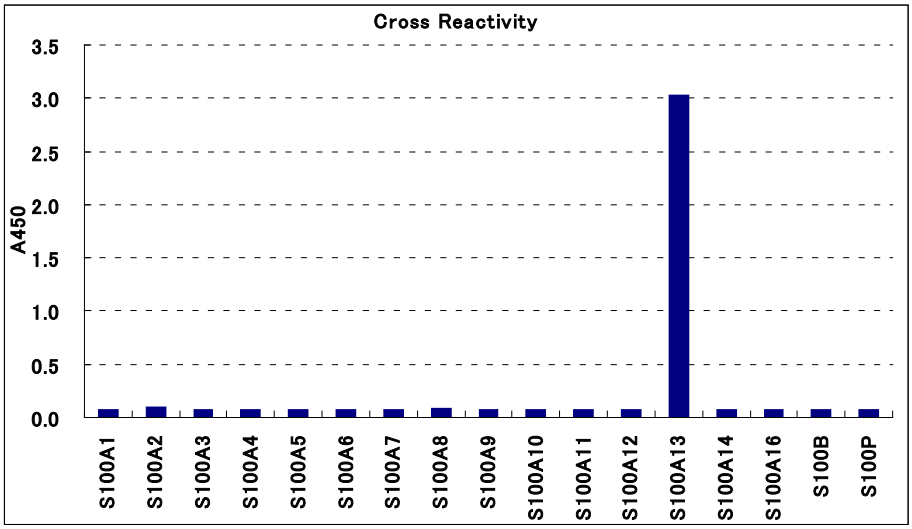
* Dilution Buffer was pipetted into blank wells.

Typical standard curve



2. Specificity

The antibodies in the CircuLex S100A13 ELISA Kit react with human S100A13 and without detectable cross-reactivities to other human S100 proteins (16 ng/ml) as indicated below.



3. Precision

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested sixteen times on one plate to assess intra-assay precision.

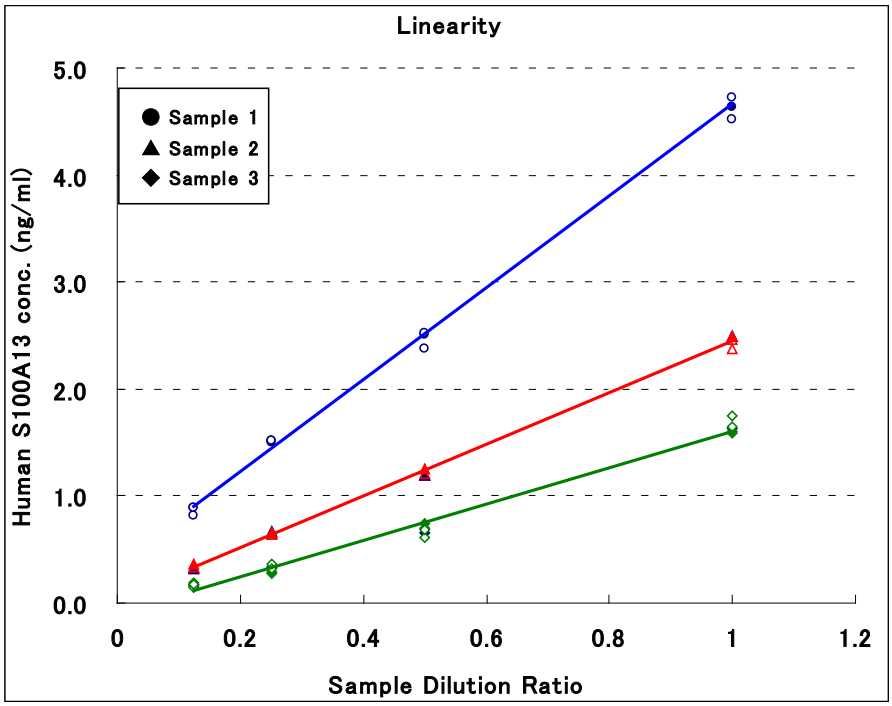
• Intra-assay (Within-Run, n=16), CV=3.4-4.8

Human S100A13 conc. (ng/mL)

	Sample 1	Sample 2	Sample 3
1	2.06	1.07	0.73
2	2.09	1.08	0.72
3	2.10	1.08	0.68
4	2.13	1.06	0.67
5	2.14	1.14	0.72
6	2.12	1.09	0.75
7	2.16	1.08	0.77
8	2.13	1.08	0.72
9	1.92	1.12	0.76
10	2.03	1.12	0.73
11	1.96	1.05	0.68
12	1.99	1.01	0.73
13	2.01	1.02	0.69
14	1.94	1.05	0.77
15	1.97	1.09	0.75
16	1.96	1.04	0.79
MAX.	**2.16**	**1.14**	**0.79**
MIN.	**1.92**	**1.01**	**0.67**
MEAN	**2.04**	**1.07**	**0.73**
S.D.	**0.08**	**0.04**	**0.03**
C.V.	**4.0%**	**3.4%**	**4.8%**

4. Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human S100A13 were serially diluted with the Dilution Buffer to produce samples with values within the dynamic range of the assay.



Example of Test Results

Fig.1 Human S100A13 concentration in several cell culture supernatants

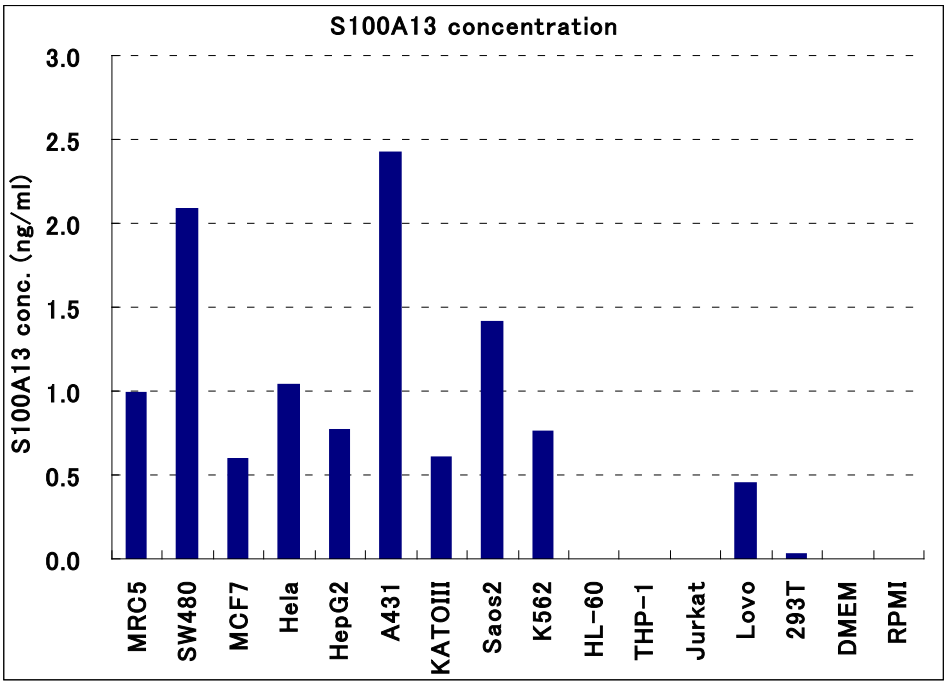
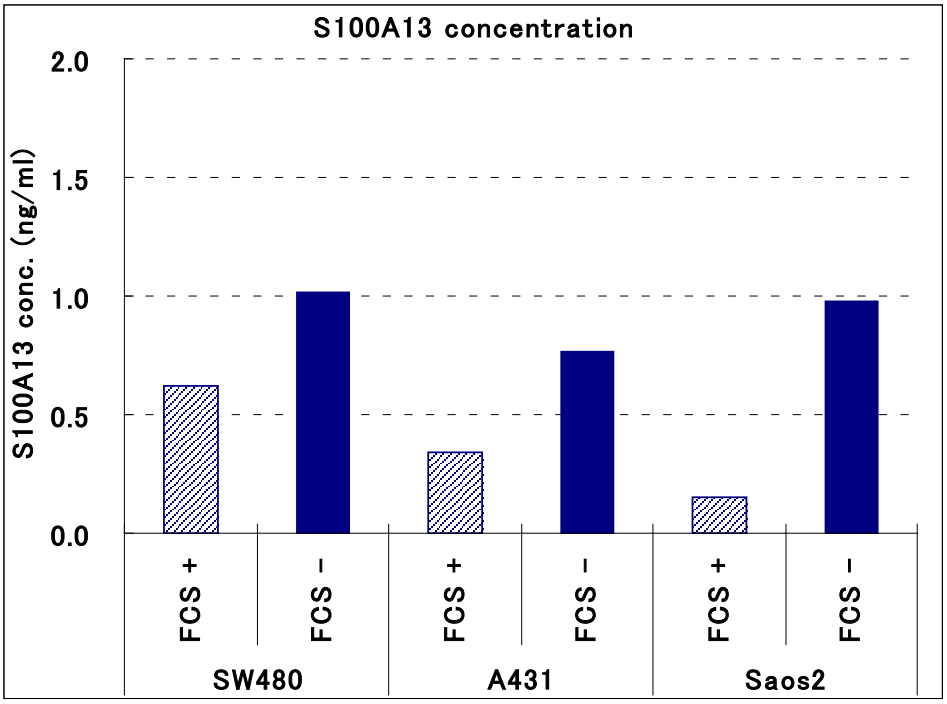


Fig.2 Effect of serum starvation on secretion of human S100A13 in several cell lines



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Related Products

*** CircuLex S100A13 ELISA Kit: Cat# CY-8057**
*** CircuLex S100A12 ELISA Kit: Cat# CY-8058**
*** CircuLex S100P ELISA Kit: Cat# CY-8060**
*** CircuLex S100A8-MRP8 ELISA Kit: Cat# CY-8061**
*** CircuLex S100A9-MRP14 ELISA Kit: Cat# CY-8062**
*** CircuLex S100A11 ELISA Kit: Cat# CY-8063**
*** CircuLex S100A14 ELISA Kit: Cat# CY-8064**
*** CircuLex S100A7/Psoriasin ELISA Kit: Cat# CY-8073**
*** CircuLex S100A4 ELISA Kit Ver.2: Cat# CY-8086**

*** Anti-Human S100A3 (Clone YK-3E3): Cat# CY-M1039**
*** Anti-Human S100A4 (p9Ka): Cat# CY-P1026**
*** Anti-Human S100P: Cat# CY-P1028**
*** Anti-Human S100A10: Cat# CY-P1033**
*** Anti-Human S100A16: Cat# CY-P1034**
*** Anti-Human S100A3: Cat# CY-P1039**
*** Anti-Human S100A2: Cat# CY-P1040**

*** Human S100B: Cat# CY-R2250**
*** Human S100A1: Cat# CY-R2251**
*** Human S100A2: Cat# CY-R2252**
*** Human S100A3: Cat# CY-R2253**
*** Human S100A4: Cat# CY-R2254**
*** Human S100A5: Cat# CY-R2255**
*** Human S100A6: Cat# CY-R2256**
*** Human S100A7: Cat# CY-R2257**
*** Human S100A8: Cat# CY-R2258**
*** Human S100A9: Cat# CY-R2259-G**

*** Human S100A9: Cat# CY-R2259-H**
*** Human S100A10: Cat# CY-R2260**
*** Human S100A12: Cat# CY-R2262-G**
*** Human S100A12: Cat# CY-R2262-H**
*** Human S100A13: Cat# CY-R2263**
*** Human S100A14: Cat# CY-R2264**
*** Human S100A16: Cat# CY-R2266**
*** Human S100P: Cat# CY-R2267**
*** Human S100A11: Cat# CY-R2269**

*** Human S100A1 Low Endotoxin: Cat# CY-R2451**
*** Human S100A3 Low Endotoxin: Cat# CY-R2453**
*** Human S100A4 Low Endotoxin: Cat# CY-R2454**
*** Human S100A7 Low Endotoxin: Cat# CY-R2457**
*** Human S100A8 Low Endotoxin: Cat# CY-R2458**
*** Human S100A9 Low Endotoxin: Cat# CY-R2459-G**
*** Human S100A11 Low Endotoxin: Cat# CY-R2461**
*** Human S100A12 Low Endotoxin: Cat# CY-R2462-G**
*** Human S100A14 Low Endotoxin: Cat# CY-R2464**
*** Human S100P Low Endotoxin: Cat# CY-R2467**

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