



S100A10 ELISA Kit

User's Manual

For Research Use Only, Not for use in diagnostic procedures

ELISA Kit for Measuring Human S100A10

CircuLex S100A10 ELISA Kit

Cat# CY-8095

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Intended Use

The CycLex Research Product **CircuLex S100A10 ELISA Kit** is used for the quantitative measurement of human S100A10 in cell lysate, cell culture supernatant, serum, plasma and other biological media.

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

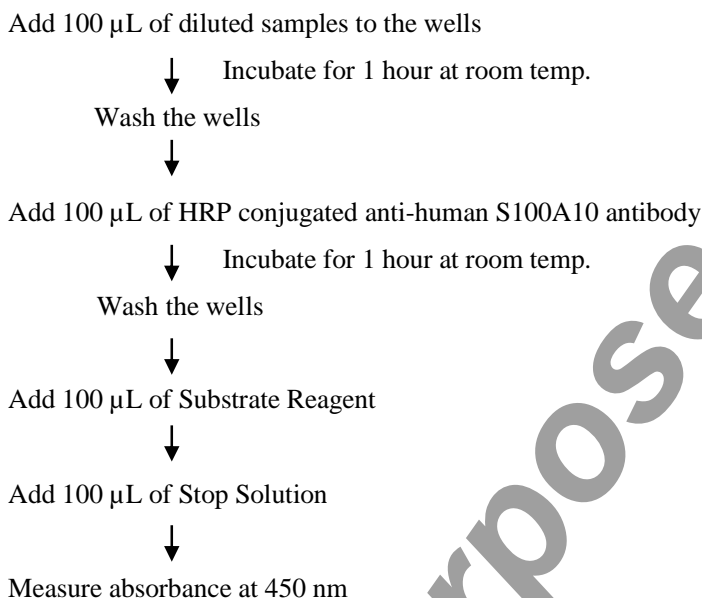
S100A10, a member of the S100 family of Ca²⁺-binding proteins, is a dimeric protein composed of two 11-kDa subunits. The protein is cytosolic when present as a dimer. Typically, S100A10 is found in most cells bound to annexin A2 as the heterotetrameric (S100A10)₂-(annexin A2)₂ complex, AII_t, in a calcium-independent manner (1). The formation of AII_t results in the translocation of S100A10 to the plasma membrane (2). S100A10 has been shown to regulate plasma membrane ion channels (3, 4) as well as cytosolic phospholipase A2 (5). In addition to an intracellular distribution, it has also been established that the heterotetrameric form of S100A10 is present on the extracellular surface of many cells (6–9). Extracellularly, the S100A10 subunit functions as a plasminogen receptor (10, 11). The penultimate and ultimate carboxyl-terminal lysines of this subunit bind tPA and plasminogen (12) and regulate the stimulation of tPA-dependent plasminogen activation (13).

Although S100A10 is expressed fairly ubiquitously in most cells and tissues, high levels of both p11 and A2 were found in 100% of anaplastic thyroid carcinomas, and correlated with their aggressive behavior (14). In a comprehensive study of S100 gene expression in over 300 primary breast cancers, both p11 (S100A10) and S100A11 were selectively upregulated in basal versus nonbasal breast cancer subtypes, but did not predict overall survival (15).

Principle of the Assay

The CycLex Research Product **CircuLex S100A10 ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human S100A10 is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human S100A10 present. After washing away any unbound substances, an HRP conjugated antibody specific for human S100A10 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 H₂O₂-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 S100A10. A standard curve is constructed by plotting absorbance values versus human S100A10 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Summary of Procedure



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-human S100A10 monoclonal antibody (YK-4G5) as a capture antibody.

10X Wash Buffer: One bottle containing 100 mL of 10X buffer containing 2% Tween®-20

Dilution Buffer: One bottle containing 50 mL of 1X buffer; use for reconstitution of Human S100A10 Standard and sample dilution. Ready to use.

Human S100A10 Standard: One vial containing 75.6 ng of lyophilized recombinant human S100A10.

HRP conjugated Detection Antibody: One bottle containing 12 mL of HRP (horseradish peroxidase) conjugated anti-human S100A10 monoclonal antibody (YK-2H4). 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 H₂SO₄. Ready to use.

Materials Required but not Provided

- **Pipettors:** 2-20 µL, 20-200 µL and 200-1000 µL precision pipettors with disposable tips
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation
- **Vortex mixer**
- **Microplate washer:** optional (Manual washing is possible but not preferable)
- **Plate reader:** capable of measuring absorbance in 96-well plates at dual wavelengths of 450/540 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.
- **Software package facilitating data generation and analysis :**optional
- **500 or 1000 mL graduated cylinder**
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

Precautions and Recommendations

- 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 residues 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 the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of human 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.

Cell lysate:

1. Harvest and pellet cells by centrifugation using standard methods.
2. Resuspend the cell pellet with an appropriate extraction buffer (for example; 20 mM HEPES-KOH, pH 7.5, 250 mM NaCl, 0.1 % NP-40, 2 mM CaCl₂, 1 mM EDTA, 0.2 mM PMSF, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 0.5 mM DTT) and lyse the resuspended cells using either a Dounce Homogenizer, sonication, or three cycles of freezing and thawing.
3. Transfer extracts to microcentrifuge tubes and centrifuge at 15,000 rpm for 10 minutes at 4°C.
4. Aliquot cleared lysate to a clean microfuge tube.
5. Assay immediately or store the samples on ice for a few hours before assaying. Aliquots of the samples may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

NOTE: Although we suggest to conduct experiments as outlined above, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user. **NO WARRANTY OR GUARANTEE OF PERFORMANCE USING THESE PROCEDURES IS MADE OR IMPLIED.**

Serum: Use a serum separator tube and allow samples to clot for 60 ± 30 minutes. Centrifuge the samples at 4°C for 10 minutes at 1,000 x g. Remove serum and assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of serum may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA-Na₂ as the anticoagulant. If possible, collect the plasma into a mixture of EDTA-Na₂ and Futhan5 to stabilize the sample against spontaneous *in vitro* complement activation. Immediately centrifuge samples at 4°C for 15 minutes at 1,000 x g. Assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of plasma may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Note: Citrate plasma has not been validated for use in this assay.

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 CycLex Research Product **CircuLex S100A10 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 S100A10 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 **S100A10 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 S100A10 Standard** with **630 µL** of **Dilution Buffer**. After dissolved by gently mixing, immediately dispense in small aliquots (e.g. 200 µL) to micro-centrifuge tubes and store at below -70°C to avoid repeated freezing and thawing. The concentration of the S100A10 in vial should be **120 ng/mL**, which is referred as a **Master Standard** of human S100A10.

Prepare Standard Solutions as follows:

Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 12 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	100 µL of Master Standard (120 ng/mL)	900 µL	12 ng/mL
Std.2	300 µL of Std. 1 (12 ng/mL)	300 µL	6 ng/mL
Std.3	300 µL of Std. 2 (6 ng/mL)	300 µL	3 ng/mL
Std.4	300 µL of Std. 3 (3 ng/mL)	300 µL	1.5 ng/mL
Std.5	300 µL of Std. 4 (1.5 ng/mL)	300 µL	0.75 ng/mL
Std.6	300 µL of Std. 5 (0.75 ng/mL)	300 µL	0.375 ng/mL
Std.7	300 µL of Std. 6 (0.38 ng/mL)	300 µL	0.188 ng/mL
Blank		300 µL	0 ng/mL

Note: Do not use a Repeating pipette. Change tips for every dilution. Unused portions of Master Standard should be aliquoted and stored at below -70°C immediately. Avoid multiple freeze and thaw cycles.

Sample Preparation

- Cell lysates and other biological samples require 10- to 50-fold dilution.
- Cell culture supernatant samples require neat to several-fold dilution.

Standard Assay Procedure for S100A10

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 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 1 hour, 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 1 hour, 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. Covering the plate with e.g. aluminum foil is recommended. 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 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 0.25 units for the blank (zero concentration), or 3.0 units for the highest standard concentration. The plate should be monitored at 5-minute intervals for approximately 30 minutes.

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 the S100A10 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 S100A10 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 S100A10 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 4-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4-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 4-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 0.188 ng/mL to 12 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 S100A10 concentration.

Troubleshooting

1. The S100A10 Standard should be run 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 CycLex Research Product **CircuLex S100A10 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 S100A10 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.

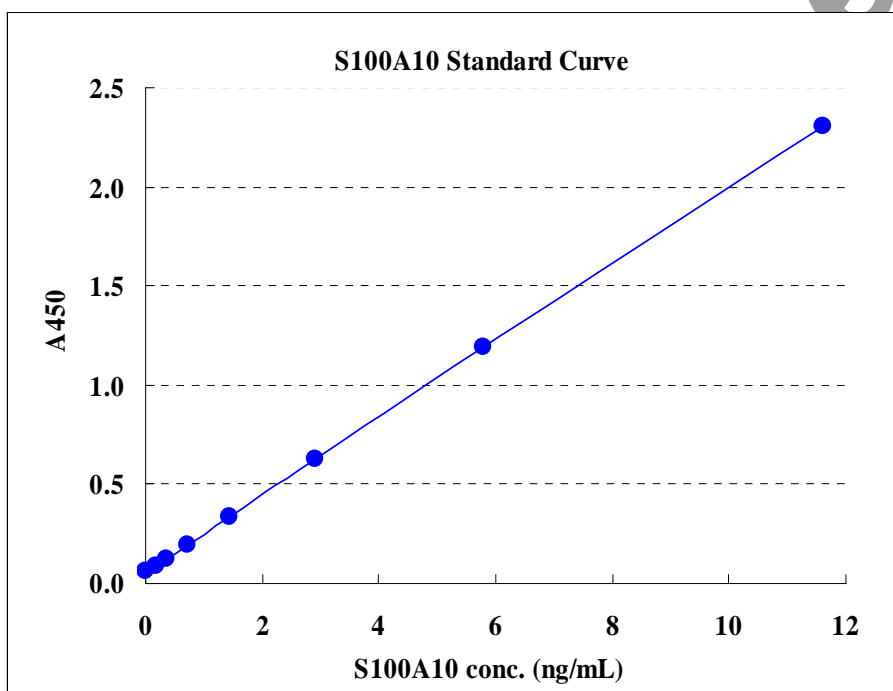
Assay Characteristics

1. Sensitivity

The limit of detection (defined as such a concentration of human S100A10 giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 82.6 pg/ml of sample.

* Dilution Buffer is pipetted into blank wells.

Typical Standard Curve



2. 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=1.3-2.9 %

*Sample: cell lysate

S100A10 conc. (ng/ml)			
	Sample 1	Sample 2	Sample 3
1	1.97	3.81	8.56
2	1.97	3.63	8.41
3	2.15	3.69	8.53
4	2.10	3.73	8.62
5	2.13	3.92	8.52
6	2.13	3.83	8.67
7	2.14	3.77	8.63
8	2.10	3.88	8.53
9	2.01	3.85	8.56
10	2.05	3.72	8.35
11	2.04	3.83	8.64
12	2.09	3.79	8.67
13	2.11	3.97	8.76
14	2.10	3.88	8.46
15	2.12	3.96	8.63
16	2.15	4.02	8.75
MAX.	2.1	4.0	8.8
MIN.	2.0	3.6	8.4
MEAN	2.1	3.8	8.6
S.D.	0.1	0.1	0.1
C.V.	2.9%	2.8%	1.3%

Inter-assay Precision (Precision between assays)

Three samples* of known concentration were tested in five separate assays to assess inter-assay precision.

- Inter-assay (Run-to-Run, n=5) CV=4.55-6.62 %

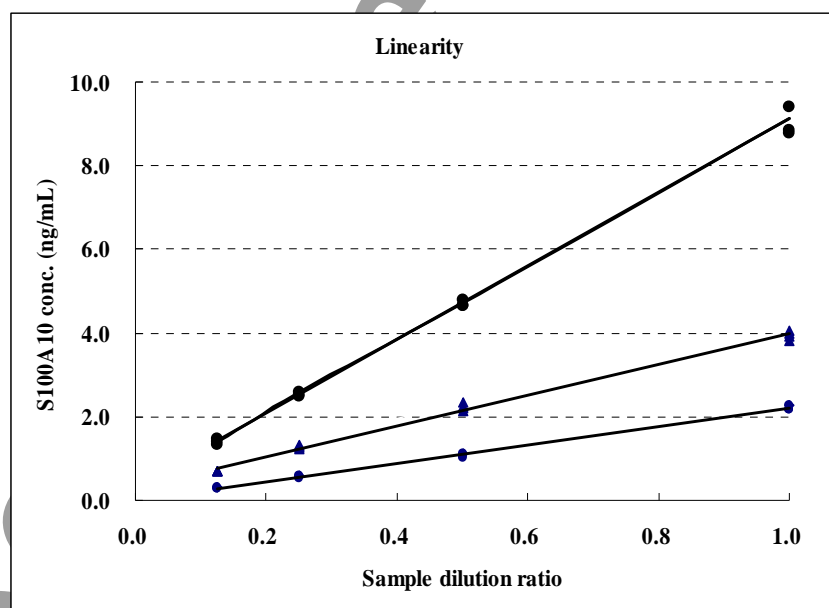
*Sample: cell lysate

	S100A10 conc. (ng/ml)		
	Sample 1	Sample 2	Sample 3
1	2.11	3.75	8.40
2	2.05	3.73	8.04
3	2.22	4.08	8.65
4	2.31	4.19	8.76
5	2.38	4.33	9.08
MAX.	2.38	4.33	9.08
MIN.	2.05	3.73	8.04
MEAN	2.21	4.01	8.59
S.D.	0.138	0.266	0.391
C.V.	6.25%	6.62%	4.55%

3. Linearity

Three biological samples* were diluted with Dilution Buffer and assayed after dilution. The neat sample is set to 1. Please note that all samples including the neat sample are 10-fold diluted as stated in the Assay Procedure. The results are summarized in the figure below.

*Sample: cell lysate



Example of Test Results

Fig.1 S100A10 concentrations in culture supernatants of several cell lines

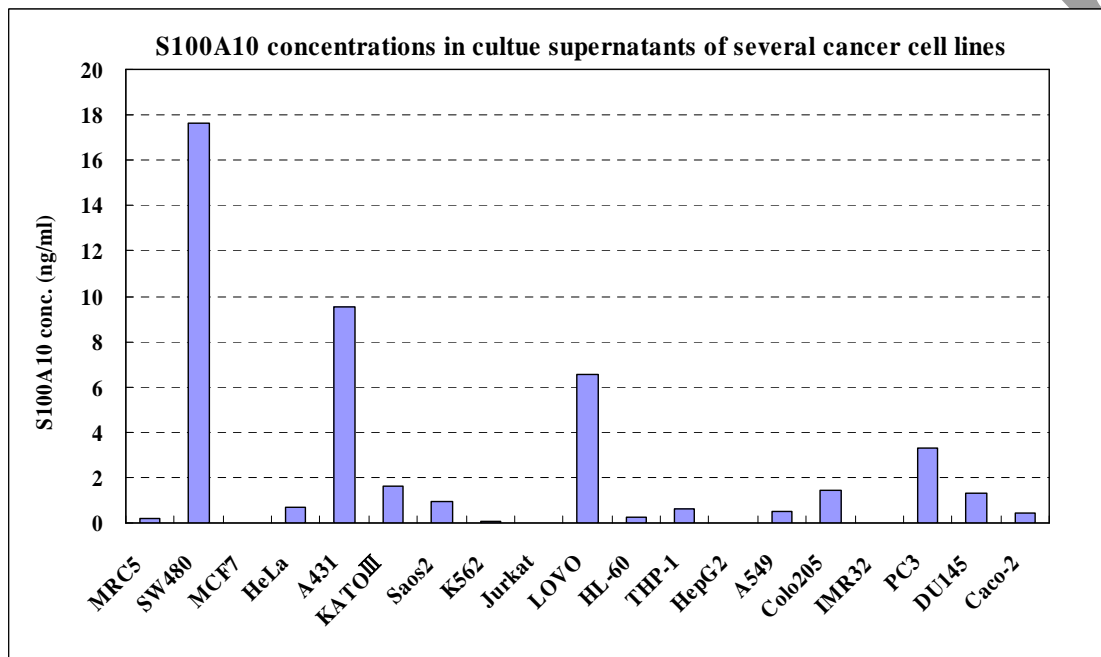
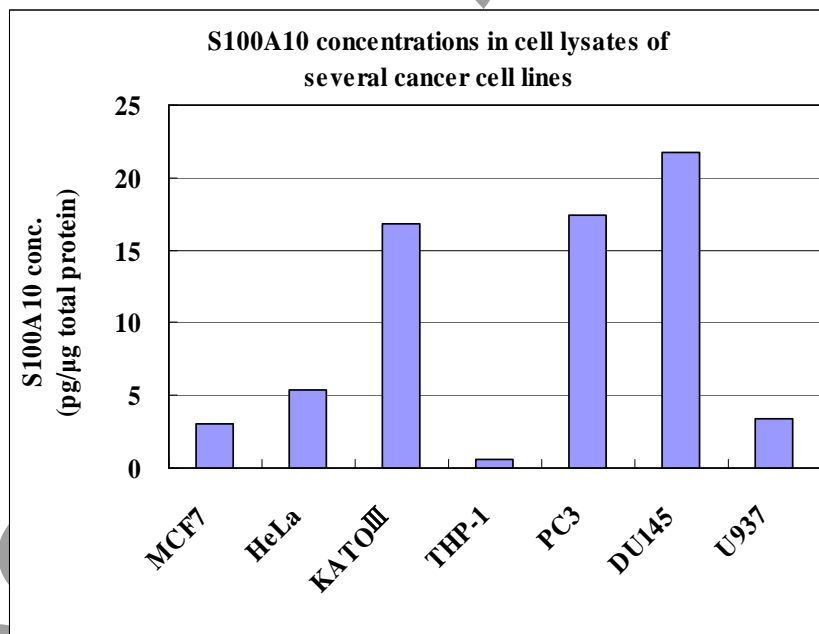


Fig.2 S100A10 concentrations in cell lysates of several cancer cell lines



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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
- * CircuLex S100A10 ELISA Kit: Cat# CY-8095

- * 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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User's Manual

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