ELISA Kit for Measuring Human S100P

CircuLex S100P ELISA Kit

Cat# CY-8060

Intended Use... 1
Storage.. 1
Introduction..2
Principle of the Assay.................................2-3
Materials Provided......................................3
Materials Required but not Provided...........4
Precautions and Recommendations............. 5
Sample Collection and Storage....................6
Detailed Protocol.. 7-8
Calculations..9
Measurement Range....................................9
Troubleshooting.. 9
Reagent Stability..9
Assay Characteristics..................................10-12
Example of Test Results............................. 12
References..13

Intended Use

The MBL Research Product **Circulex S100P ELISA Kit** is used for the quantitative measurement of human S100P in cell culture supernatant, cell lysate and serum.

Individual users should determine appropriate conditions when using other types of 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.

Copy on Web. Just For Reference Purpose Only.

Introduction

Members of the S100 protein family are low molecular mass acidic proteins characterized by cell-type-specific expression and the presence of 2 EF-hand calcium-binding domains. One of the least studied members of the S100 family is S100P, a 95-amino acid protein first purified from placenta with a restricted cellular distribution (1, 2). The molecular structure of S100P has been well described and supports its classification in the S100 family of proteins (3). Expression of S100P has been noted in esophageal epithelial cells during their differentiation, indicating that it may play a role in normal development (4). There is also considerable evidence that S100P plays a role in cancer. S100P expression has been noted in various cancer cell lines including breast cancer, where it was associated with cellular immortalization (5), and colon cancer, where its expression was elevated in doxorubicin-resistant cells (6). S100P has also been shown to be expressed in tumors, including prostate cancer, where its expression is androgen-sensitive (7), and pancreatic adenocarcinoma, where expression has been localized to the neoplastic epithelium of pancreatic (8). Furthermore, S100P expression has been shown to be correlated with decreased survival in patients with lung cancer (9).

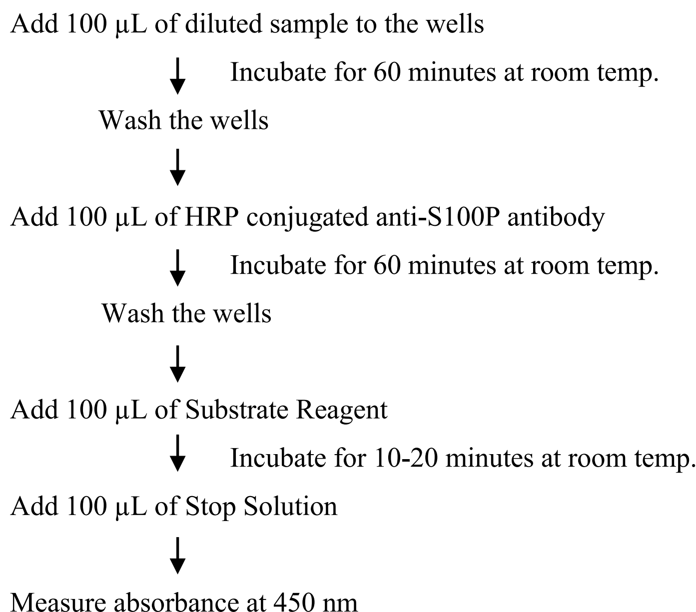
Principle of the Assay

The Circulex S100P ELISA Kit employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for S100P has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any S100P present. After washing away any unbound substances, an HRP conjugated antibody specific for S100P 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 S100P. A standard curve is constructed by plotting absorbance values versus S100P concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

The Circulex S100P ELISA Kit is designed to measure the concentration of S100P from cell lysate of cultured human cell lines, cell culture conditioned medium and human serum/plasma.

Copy on Web. Just For Reference Purpose Only.

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-S100P antibody as a capture antibody.

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

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

Human S100P Standard: One vial containing X* ng of lyophilized recombinant human S100P
***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-S100P 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.

Copy on Web. Just For Reference Purpose Only.

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**

Copy on Web. Just For Reference Purpose Only.

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 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.

Copy on Web. Just For Reference Purpose Only.

Sample Collection and Storage

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 Tris-HCl, pH 7.5, 250 mM NaCl, 0.5 % NP-40, 1 mM EDTA, 1 mM PMSF, 10 µM aprotinin) 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.

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.

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 -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

Other biological samples: MBL has not tested.
(*e.g.* Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles. Individual users should determine appropriate conditions when using other types of samples.)

Copy on Web. Just For Reference Purpose Only.

Detailed Protocol

The MBL Research Product **Circulex S100P 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 S100P 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 S100P 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 (ddH_2O). Mix well.

2. Reconstitute **Human S100P Standard** with X* **µL** of **Dilution Buffer**. The concentration of the human S100P in vial should be **10 ng/mL**, which is referred as a **Master Standard** of human S100P.
   ***The amount is changed depending on lot. See the real "User's Manual" included in the kit box.**

   Prepare Standard Solutions as follows:
   Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2,500 pg/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	2,500 pg/mL
Std.2	300 µL of Std. 1 (2,500 pg /mL)	300 µL	1,250 pg/mL
Std.3	300 µL of Std. 2 (1,250 pg/mL)	300 µL	625 pg/mL
Std.4	300 µL of Std. 3 (625 pg/mL)	300 µL	312.5 pg/mL
Std.5	300 µL of Std. 4 (312.5 pg/mL)	300 µL	156.3 pg/mL
Std.6	300 µL of Std. 5 (156.3 pg/mL)	300 µL	78.1 pg/mL
Std.7	300 µL of Std. 6 (78.1 pg/mL)	300 µL	39.0 pg/mL
Blank	-	300 µL	0 pg/mL

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

Sample Preparation

Dilute samples with **Dilution Buffer**.

- Serum samples may require a 10-fold dilution.
  e.g. 30 µL sample + 270 µL Dilution Buffer
- Culture supernatant and cell lysate may require no dilution or appropriate dilution.

Copy on Web. Just For Reference Purpose Only.

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 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. 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 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 S100P concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Copy on Web. Just For Reference Purpose Only.

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 S100P 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 S100P 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 39 pg/mL to 2,500 pg/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 S100P concentration.

Troubleshooting

1. All samples and standards 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. <u>Do not allow the plate to dry out</u>. Add Substrate Reagent immediately after wash.

Reagent Stability

All of the reagents included in the MBL Research Product **Circulex S100P 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 S100P 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.

Copy on Web. Just For Reference Purpose Only.

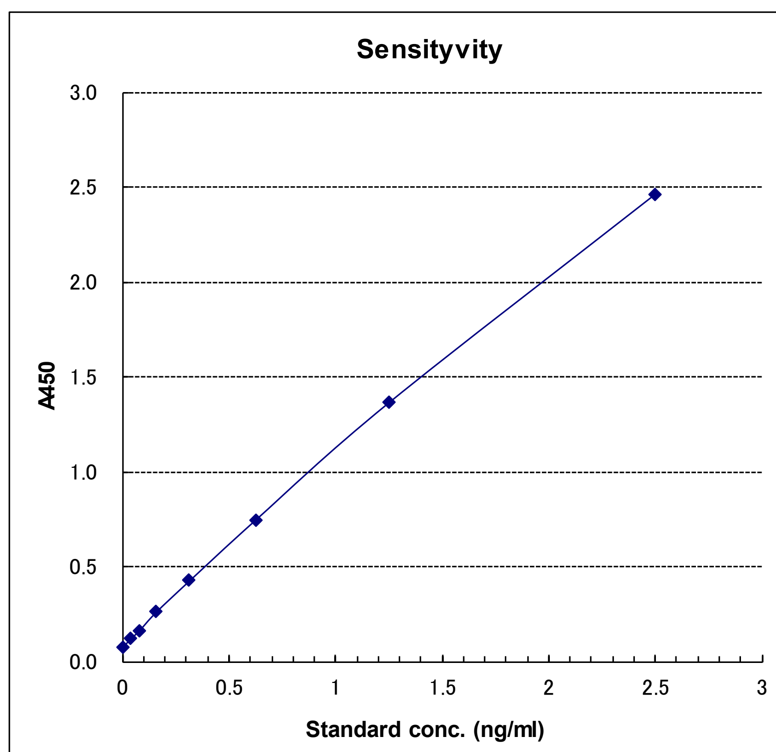
Assay Characteristics

1. Sensitivity

The limit of detection (defined as such a concentration of S100P giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 29 pg/mL of sample.

* Dilution Buffer was into blank wells.

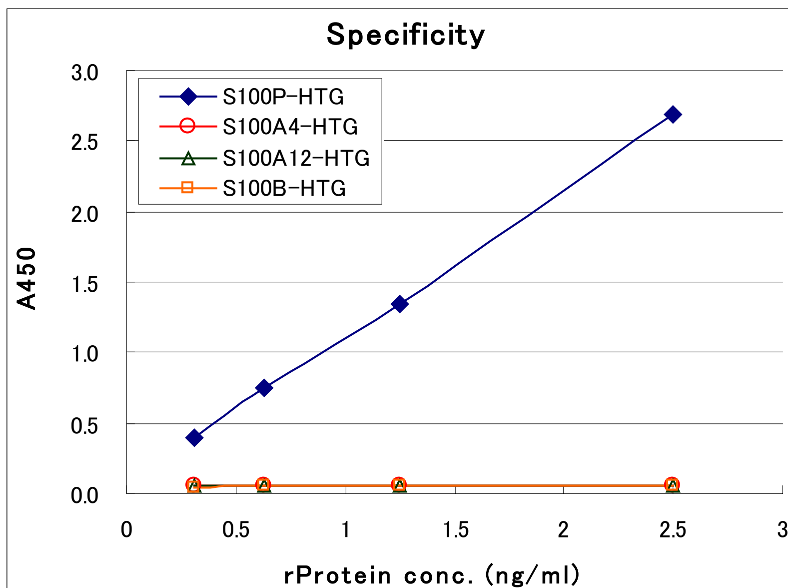
Eighty assays were evaluated and the minimum detectable dose (MDD) of S100P ranged from 21.5-34.3 pg/mL. The mean MDD was 28.8 pg/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.



Copy on Web. Just For Reference Purpose Only.

2. Specificity

The antibodies in the Circulex S100P ELISA Kit are highly specific of S100P, with no detectable cross-reactivity to S100A12, S100A4 and S100B. Cross-reactivities to other S100 family member have not checked yet.



3. Precision

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

- Intra-assay (Within-Run, n=8) CV = 4.5 %

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

- Inter-assay (Run-to-Run, n=4) CV = 6.6 %

4. Spiking Recover

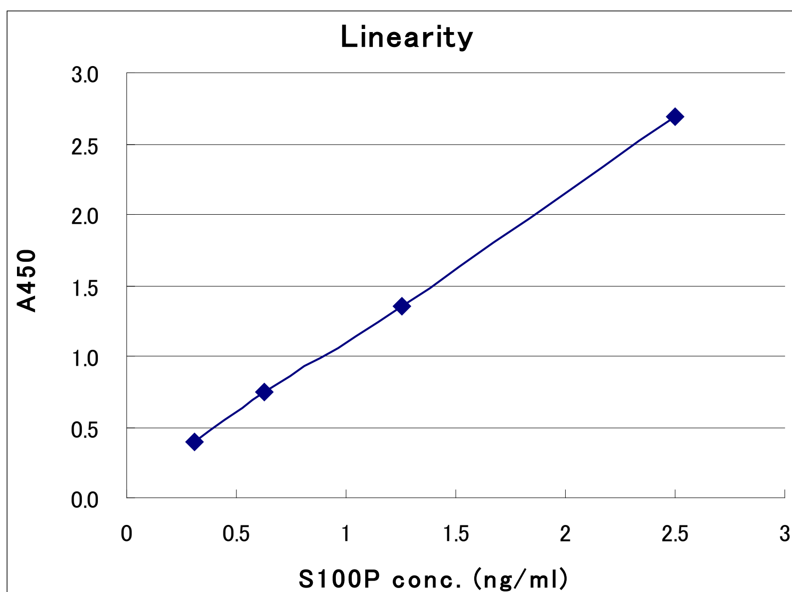
Serum samples were spiked with different amounts of S100P and assayed.
The recovery of S100P spiked to levels throughout the range of the assay was evaluated.
Sample Average % Recovery Range
Cell culture media (n=4)

- Recovery = 109, 85, 97, 115

Copy on Web. Just For Reference Purpose Only.

5. Linearity

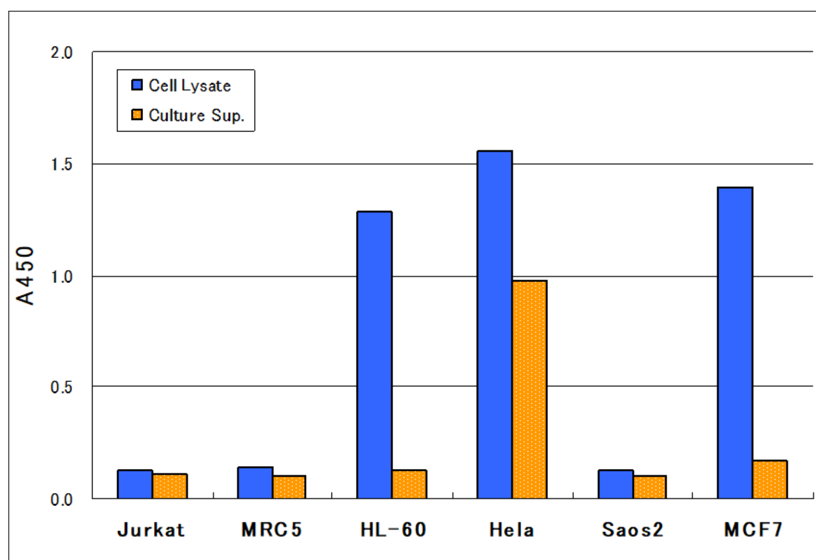
To assess the linearity of the assay, samples containing and/or spiked with high concentrations of S100P were serially diluted with the Dilution Buffer to produce samples with values within the dynamic range of the assay.



Example of Test Results

Fig.1 S100P level in culture supernatant and cell lysate of several cell lines

Measured S100P level in Culture supernatant and Cell lysate.
Dilute Culture supernatant 80% confluent cultured cell 2-fold with dilution buffer.
1ml of Cell lysate prepared from 80% confluent cell cultured in 75cm^2 flask or 10cm diameter dish.



Copy on Web. Just For Reference Purpose Only.

References

1. Becker, T., Gerke, V., Kube, E., and Weber, K. (1992) Eur. J. Biochem. 207, 541–547

2. Emoto, Y., Kobayashi, R., Akatsuka, H., and Hidaka, H. (1992) Biochem. Biophys. Res. Commun. 182, 1246–1253

3. Zhang, H., Wang, G., Ding, Y., Wang, Z., Barraclough, R., Rudland, P. S., Fernig, D. G., and Rao, Z. (2003) J. Mol. Biol. 325, 785–794

4. Sato, N., and Hitomi, J. (2002) Anat. Rec. 267, 60–69

5. Guerreiro, D. S., I, Hu, Y. F., Russo, I. H., Ao, X., Salicioni, A. M., Yang, X., and Russo, J. (2000) Int. J. Oncol. 16, 231–240

6. Bertram, J., Palfner, K., Hiddemann, W., and Kneba, M. (1998) Anticancer Drugs 9, 311–317

7. Averboukh, L., Liang, P., Kantoff, P. W., and Pardee, A. B. (1996) Prostate 29, 350–355

8. Logsdon, C. D., Simeone, D. M., Binkley, C., Arumugam, T., Greenson, J. K., Giordano, T. J., Misek, D. E., and Hanash, S. (2003) Cancer Res. 63, 2649–2657

9. Beer, D. G., Kardia, S. L., Huang, C. C., Giordano, T. J., Levin, A. M., Misek, D. E., Lin, L., Chen, G., Gharib, T. G., Thomas, D. G., Lizyness, M. L., Kuick, R., Hayasaka, S., Taylor, J. M., Iannettoni, M. D., Orringer, M. B., and Hanash, S. (2002) Nat. Med. 8, 816–824

For more information, please visit our website at
https://ruo.mbl.co.jp/.

MANUFACTURED BY

URL: https://ruo.mbl.co.jp
E-mail: support@mbl.co.jp

CycLex/CircuLex products are supplied for research use only. CycLex/CircuLex products and components thereof may not be resold, modified for resale, or used to manufacture commercial products without prior written approval from MBL. To inquire about licensing for such commercial use, please contact us via email.

Copy on Web. Just For Reference Purpose Only.