Human S100A6 ELISA KIT

Cat. No.:DEIA7079
Pkg.Size:96T

Intended use

The Human S100A6 ELISA KIT is suitable for the quantitative determination of human S100A6 concentrations in serum and plasma.

General Description

Protein S100-A6 is a protein that in humans is encoded by the S100A6 gene.
The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100 genes include at least 13 members which are located as a cluster on chromosome 1q21. This protein may function in stimulation of Ca2+-dependent insulin release, stimulation of prolactin secretion, and exocytosis. Chromosomal rearrangements and altered expression of this gene have been implicated in melanoma.

Principle Of The Test

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for S100A6 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any S100A6 present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for S100A6 is added to the wells. Following a wash to remove any unbound antibody reagent, an Anti Rabbit IgG HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of S100A6 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. S100A6 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a purified monoclonal IgG against S100A6. 1 plate
2. S100A6 Standard - 50 ng/vial of recombinant Human S100A6 in a buffered protein base with preservatives; lyophilized. 1 vial
3. Detection Antibody - 1.05 mL/vial, 10-fold Concentrate of a purified polyclonal IgG against S100A6 with preservatives; lyophilized. 1 vial
4. Positive Control - one vial of recombinant S100A6 , lyophilized. 1 vial
5. Anti Rabbit IgG-HRP Conjugate - 120 µL/vial, 100- fold concentrated solution of Goat anti Rabbit IgG conjugate to HRP. 1 vial
6. Dilution Buffer - 60mL of buffered protein based solution with preservatives. 1 bottle
7. HRP Diluent Solution - 12mL of buffered protein based solution with preservatives. 1 bottle
8. Wash Buffer - 50mL of 10-fold concentrated buffered surfactant, with preservative. 1 bottle
9. TMB Substrate Solution - 11mL of TMB substrate solution. 1 bottle
10. Stop Solution - 11mL of 0.5M HCl. 1 bottle
11. Plate Sealer. 1
12. Plastic Pouch. 1

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Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. Microplate shaker (250-300rpm).
3. Pipettes and pipette tips.
4. Deionized or distilled water.
5. Squirt bottle, manifold dispenser, or automated microplate washer.
6. 100 mL and 500 mL graduated cylinders.

Storage

Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Anti Rabbit IgG-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 12 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

Specimen Collection And Handling

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.
SAMPLE PREPARATION
Serum and plasma samples may require dilution. Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

Reagent Preparation

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
S100A6 Standard - Refer to vial label for reconstitution volume. Reconstitute the S100A6 standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 50 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 50 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).
Table 1.

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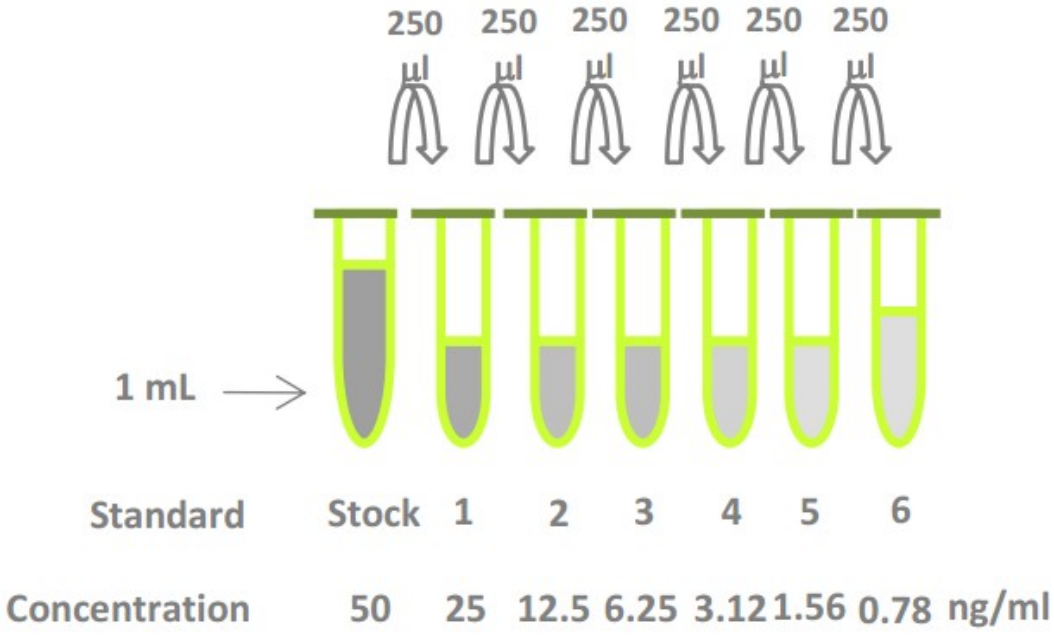
TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	50 ng/ml
# 1	250µl of stock	250µl	25 ng/ml
# 2	250µl of 1	250µl	12.5 ng/ml
# 3	250µl of 2	250µl	6.25 ng/ml
# 4	250µl of 3	250µl	3.125 ng/ml
# 5	250µl of 4	250µl	1.56 ng/ml
# 6	250µl of 5	250µl	0.78 ng/ml

Figure 1.



Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the appropriate Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Anti Rabbit IgG-HRP Conjugate - Transfer 120 µL of 100-fold concentrated Anti Rabbit IgG-HRP Conjugate stock solution to 11.88 mL of HRP Diluent Solution (DB08) to prepare working solution. Note: 1x working solution of Anti Rabbit IgG-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the Positive Control with 1.5 mL of Dilution Buffer. Note: Positive Control should be prepared and used immediately.

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Assay Steps

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal.
3. Add 100 µL of Dilution Buffer to Blank wells (A2, A3).
4. Add 100 µL of Standard solution from #6 to #S (reverse order of serial dilution) (from H2, H3 to B2, B3), sample, or positive control (H4, H5) per well. Cover with the plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. 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.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with the plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Anti Rabbit IgG-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. Protect from light.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 15 minute at room temperature. Protect from light. Be ready to add stop solution quickly.
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

Figure 2:

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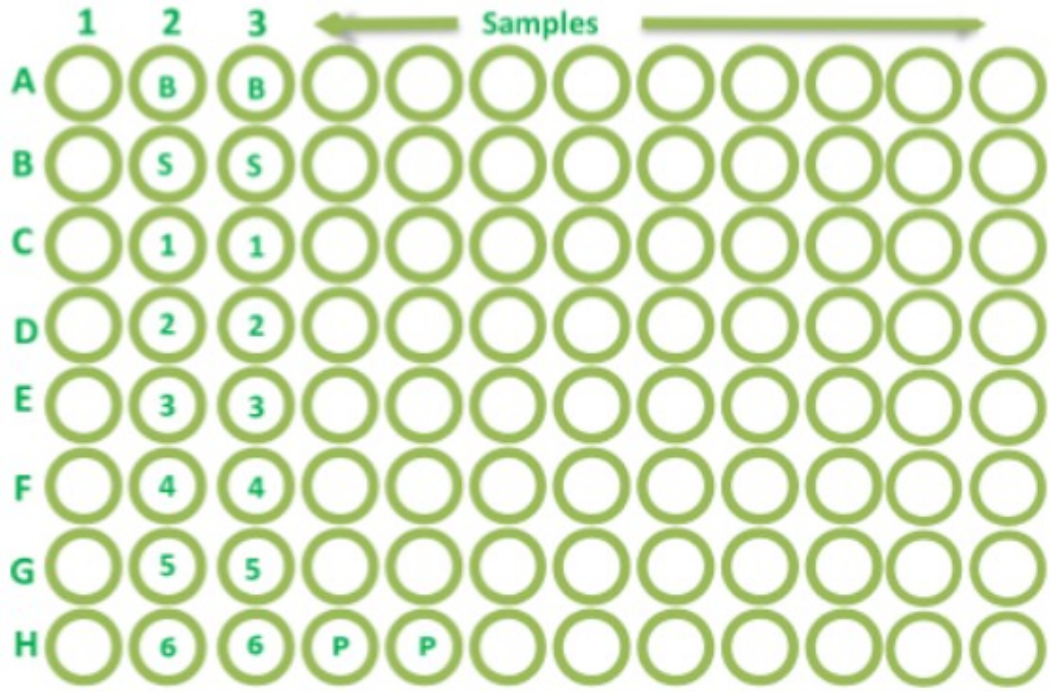

Calculation

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the S100A6 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Calculation of samples with a concentration exceeding that of 50 ng/mL may result in inaccurate, low human S100A6 levels. Such samples require further external predilution according to expected human S100A6 values with Dilution Buffer in order to precisely quantify the actual human S100A6 level.

Typical Standard Curve

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.
• Positive control: 15 - 30 ng/mL
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STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.105)
1.56	0.117
3.12	0.140
6.25	0.280
12.5	0.437
25	0.655
50	0.937
100	1.102

Evaluation

This immunoassay is calibrated against a highly purified recombinant Human S100A6.

Detection Range

0.78-50 ng/mL

Sensitivity

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of S100A6 was 200 pg/mL.

Specificity

This assay recognizes both natural and recombinant human S100A6. The factors listed below were prepared at 5000 ng/mL in Dilution Buffer and assayed for cross reactivity.
Table 3:

PROTEIN NAME	CROSS-REACTIVITY
Human S100A6	100%
Human Fetuin A	0

Reproducibility

Intra-assay Precision: 4-6%
Inter-assay Precision: 8-10%

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Precautions

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

Limitations

1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
4. If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
5. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
6. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

Analyte Gene Information

Gene Name	S100A6 S100 calcium binding protein A6 [Homo sapiens]
Official Symbol	S100A6
Synonyms	S100A6; S100 calcium binding protein A6; CACY, S100 calcium binding protein A6 (calcyclin) , S100 calcium binding protein A6 (calcyclin); protein S100-A6; 2A9; CABP; PRA; MLN 4; calcyclin; growth factor-inducible protein 2A9; prolactin receptor-associated protein; S100 calcium-binding protein A6 (calcyclin); 5B10; CACY;
GeneID	6277
mRNA Refseq	NM_014624
Protein Refseq	NP_055439
MIM	114110
UniProt ID	P06703
Chromosome Location	1q21
Pathway	Prostaglandin Synthesis and Regulation, organism-specific biosystem;
Function	S100 beta binding; calcium ion binding; calcium ion binding; calcium-dependent protein binding; ion transmembrane transporter activity; protein binding; protein homodimerization activity; protein homodimerization activity; tropomyosin binding; zinc ion binding;

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