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DESCRIPTION

Human S100A8/S100A9 Heterodimer Immunoassay

Catalog Number:SEKH-0423

For the quantitative determination of human S100A8/S100A9 Heterodime concentrations in cell culture supernates, serum, and

For research use only. Not for use in diagnostic procedures.

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LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of S100A8/S100A9 Heterodimerin in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	105	103
	Range(%)	95—117	93—112
1:4	Average% of Expected	98	104
	Range(%)	89—110	92—113

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 31 pg/mL
SPECIFICITY: This assay recognizes both natural and recombinant human S100A8/S100A9 Heterodimer. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant rat
RAGE/Fc Chimera	S100A8/S100A9 Heterodimer	
S100A4		
S100A8		
S100A9		
S100A13		
S100B		
TLR4		
TLR4/MD-2 Complex		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
RECOVERY: The recovery of S100A8/S100A9 Heterodimer spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of S100A8/S100A9 Heterodimer in two matrices

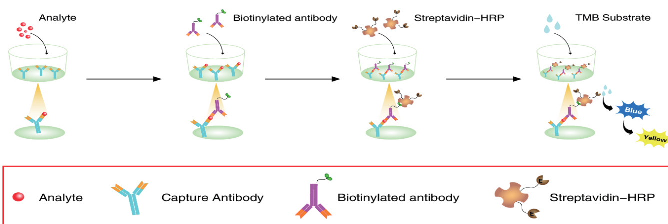
Sample Type	Average % of Expected Range(%)	Range(%)
Citrate plasma	97	86—105
Cell culture supernatants	101	95—110

BACKGROUND

S100A8 (also MRP8 and calgranulin A) is a 10 kDa member of the S100 family, EF-hand superfamily of Ca-binding proteins. It is produced by neutrophils and monocytes, and forms Ca2+-dependent heterodimer/heterotetramer complexes (termed calprotectin) with S100A9. It functions both intracellularly and extracellularly, where it binds to RAGE and CD36.
Human S100A8 is 93 amino acids (aa) in length. It contains two EF-hand motifs (aa 12 - 47 and 46 - 81) and one high-affinity Ca2+-binding site (aa 59 - 70). There may be one splice form that shows a 15 aa substitution for the C-terminal 14 amino acids. Although mouse S100A8 is cleaved by MMP-2 after Asn21, it is unclear if human S100A8 is susceptible.
Full-length human S100A8 is 57% and 74% aa identical to mouse and canine S100A8, respectively.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for S100A8/S100A9 Heterodimer has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any S100A8/S100A9 Heterodimer present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for S100A8/S100A9 Heterodimer is added to detect the captured S100A8/S100A9 Heterodimer protein in sample. For signal development, horseradish peroxidase (HRP)-conjugatedStreptavidinis added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional



TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

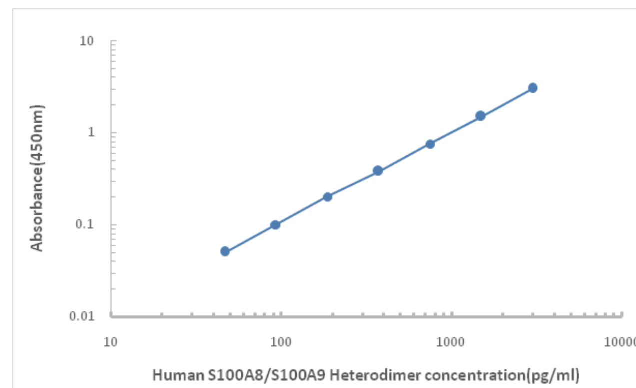
PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

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
5.multiplied by the dilution factor.
This standard curve is provided for demonstration only. A standard

Typical data using the S100A8/S100A9 Heterodimer ELISA

Standared(pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.057	0.053	--
46.88	0.182	0.188	0.185	0.132
93.8	0.265	0.273	0.269	0.216
188	0.394	0.406	0.400	0.348
375	0.626	0.645	0.636	0.583
750	1.017	1.048	1.032	0.980
1500	1.655	1.706	1.681	1.628
3000	2.697	2.780	2.738	2.686



Representative standard curve for S100A8/S100A9 Heterodimer ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed,wash the plate 3 times before the assay.

Add 100μl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-humanS100A8/S100A9 Heterodimer antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature

Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-duck IgG antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 10-30 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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.
4. The data may be linearized by plotting the log of the S100A8/S100A9 Heterodimer concentrations versus the log of the O.D. and the best fit

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack.Reseal along entire edge of the zip-seal.May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,3000 pg/ml upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

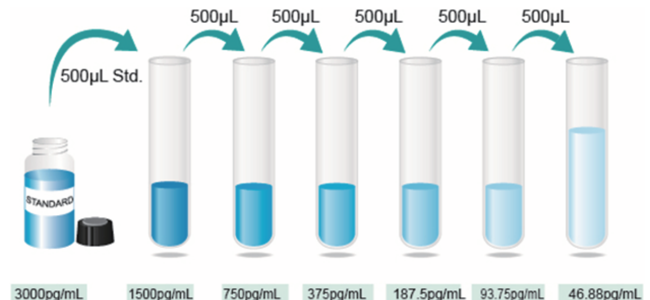
SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃.Centrifuge approximately for 15 minutes at 1000g(or 3000rpm). 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 1000g(or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning -** Bring all kit components and specimen to room temperature(20-25℃) before use.
2. **Wash Buffer -** Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard**Sample - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 3000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.Pipette500 uL of Standard/Sample Diluent into 1500pg/ml tube and the remaining tubes. Use the stock solution of 3000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 3000pg/mL standard serves as the high standard. The Standard/ Sample Diluent serves as the zero standard (0 pg/mL).
   ***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**
4. **Working solution of Biotin-Conjugate anti-human S100A8/S100A9 Heterodimer antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**



Preparation of S100A8/S100A9 Heterodimerstandard dilutions