S100A8 (Human) ELISA Kit

Cat. No.:DEIA4161
Pkg.Size:96T

Intended use

This kit is used for the quantitative measurement of human S100A8/MRP8 in serum, plasma and other biological media.

General Description

S100A8/MRP8 and its hetero-dimerization partner S100A9/MRP14 belong to the low molecular weight calcium-binding protein, S100 family, they are composed of two distinct helix-loop-helix motifs (EF-hands) flanked by hydrophobic regions at either terminus and separated by a central hinge region. In human, 100A8 is usually co-expressed with S100A9. Both proteins are expressed during myeloid differentiation, are abundant in granulocytes and monocytes, and form heterodimeric complexes. Although a number of possible functions for S100A8-A9 heterocomplex, including antimicrobial activity, have been proposed, the exact role of these proteins in cell metabolism is still unclear. In human, they have been associated with several inflammatory diseases: elevated serum levels of S100A8 and S100A9 have been found in patients suffering from a number of inflammatory disorders including giant cell arteritis, cystic fibrosis, rheumatoid arthritis, dermatoses, chronic inflammatory bowel disease, chronic bronchitis, some malignancies and autoimmune diseases. Both proteins are localized predominantly in the cytoplasm. It was demonstrated with human monocytes that both proteins are secreted by an energy-consuming pathway, which is dependent on an intact microtubule network and involves protein kinase C. It was shown that the expression of S100A8 is up regulated by anti-inflammatory mediators and oxidative stress such as UVA and H2O2. In addition, this protein seems to be a potent scavenger of hypochlorite. Thus this protein may regulate oxidative events associated with inflammation.

Principle Of The Test

This kit employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human S100A8/MRP8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human S100A8/MRP8 present. After washing away any unbound substances, an HRP conjugated polyclonal antibody specific for human S100A8/MRP8 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 S100A8/MRP8. A standard curve is constructed by plotting absorbance values versus human S100A8/MRP8 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Reagents And 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.
1. 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-S100A8/MRP8 polyclonal antibody as a capture antibody.
2. 10X Wash Buffer: One 100 mL bottle of 10X buffer containing 2%Tween®-20
3. Dilution Buffer: One bottle containing 60 mL of 1X buffer; use for sample dilution. Ready to use.
4. Human S100A8/MRP8 Standard: Two vials containing 40 ng each of lyophilized recombinant human S100A8/MRP8

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5. HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-human S100A8/MRP8 polyclonal antibody. Ready to use.
6. Substrate Reagent: 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.
7. Stop Solution: One bottle containing 20 mL of 1 N H2SO4. Ready to use.

Materials Required But Not Supplied

1. Pipettors: 2-20 µL, 20-200 µL and 200-1000 µL precision pipettors with disposable tips.
2. Precision repeating pipettor.
3. Orbital microplate shaker
4. Microcentrifuge and tubes for sample preparation.
5. Vortex mixer.
6. Microplate washer: optional (Manual washing is possible but not preferable)
7. 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.
8. Software package facilitating data generation and analysis :optional
9. 500 or 1000 mL graduated cylinder.
10. Reagent reservoirs.
11. Deionized water of the highest quality.
12. Disposable paper towels.

Storage

All of the reagents included in the S100A7 (Human) 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 S100A7 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.

Specimen Collection And Handling

1. Washing fluid from skin: Rinse 0.5 cm2 area of various body skin with 250 µL of 10 mM sodium phosphate buffer, pH 7.4. according to Glaser, R et al.. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at -70°C. Avoid repeated freeze-thaw cycles.
2. Serum: Allow blood 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.
3. 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 -70° C for extended periods of time. Avoid repeated freeze-thaw cycles.
Note: Citrate plasma has not been validated for use in this assay.
4. Other biological samples: Remove any particulates by centrifugation and assay immediately or aliquot and store samples at -70°C. Avoid repeated freeze-thaw cycles
5.Sample Preparation
Serum and plasma samples require a 100-fold dilution e.g. 3 µL of sample + 297 µL of Dilution Buffer
Other biological samples require neat, 10- and 100-fold dilution.

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Reagent Preparation

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 S100A8 Standard.
1. Prepare a working solution of Wash Buffer by adding 100 mL of the 10X Wash Buffer (provided) to 900 mL of deionized (distilled) water. Mix well.
2. Reconstitute Human S100A8 Standard with 400 uL of Dilution Buffer. The concentration of the Human S100A8/MRP8 in vial should be 100 ng/mL, which is referred as a Master Standard of Human S100A8/MRP8.
Prepare Standard solutions as follows:
Use the Master Standard to produce a dilution series. Mix each tube thoroughly before the next transfer. The 5,000 pg/mL standard (Std.1) serves as the high standard. The Dilution Buffer serves as the zero standard (Blank).

Assay Steps

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 serum samples 1:100 with Dilution Buffer (e.g. 3 µL serum sample + 297 µL Dilution Buffer).
3. Pipette 100 µL of Human S100A8 Standards (Std1-Std7, Blank) and diluted samples in duplicates, into the appropriate wells.
4. Incubate the plate at room temperature (ca. 20°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. 20°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 A to 2-8°C immediately after the necessary volume is removed
10. Incubate the plate for 10-15 minutes at room temperature. (The incubation time may be extended up to 20 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 concentrations), or 2.5 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 Human S100A8/MRP8 concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculation

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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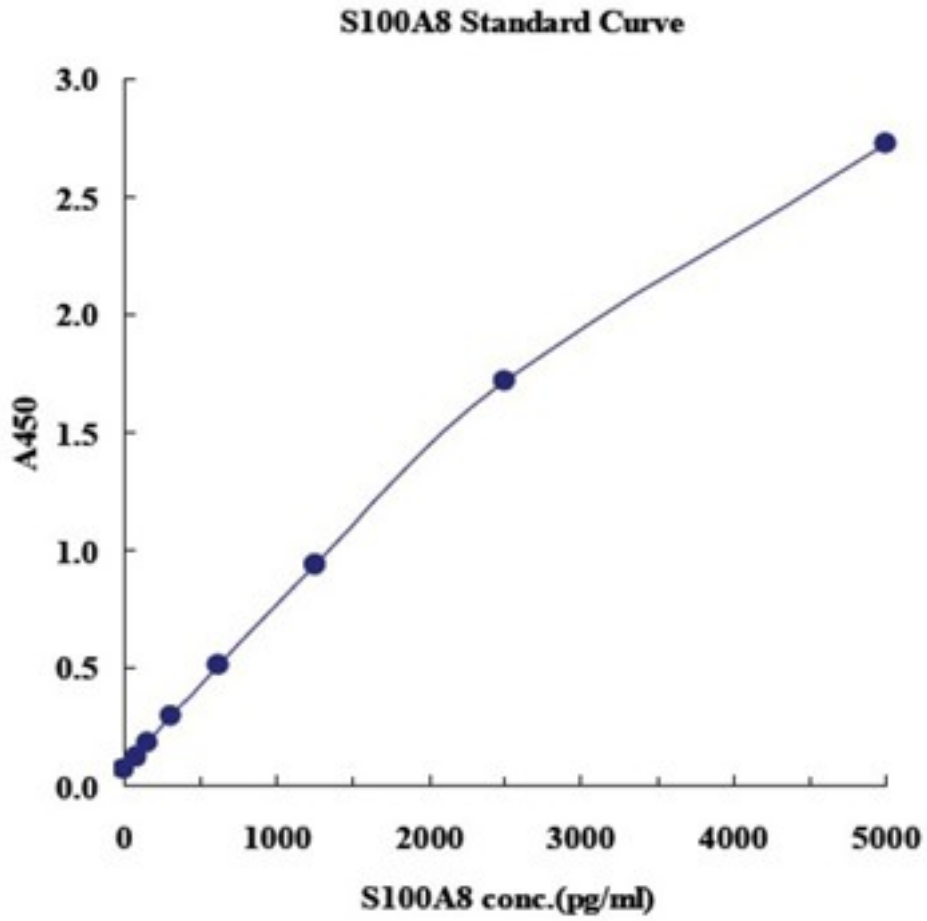
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the human S100A8/MRP8 concentration of each sample, first find 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 S100A8/MRP8 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).

Typical Standard Curve

Typical standard curve of human S100A7/Psoriasin ELISA



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Detection Range

Dilution factors need to be taken into consideration in calculating the human S100A8/MRP8 concentration. Results exceeding human S100A8/MRP8 level of 500 ng/ml should be repeated with diluted samples.

Sensitivity

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of human S100A8/MRP8. The MDD (defined as such a concentration of human S100A8/MRP8 giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 43.4 pg/ml of sample.

Specificity

The antibodies in the S100A8 (Human) ELISA Kit are highly specific of human S100A8, with no detectable cross-reactivity to all other S100 proteins.

Reproducibility

Intra-assay (Within-Run, n=16) CV=3.6 - 4.1 %
Inter-assay (Run-to-Run, n=16) CV=3.4 – 7.1 %

Analyte Gene Information

Gene Name	S100A8 S100 calcium binding protein A8 [Homo sapiens]
Official Symbol	S100A8
Synonyms	S100A8; S100 calcium binding protein A8; CAGA, CFAG, S100 calcium binding protein A8 (calgranulin A) , S100 calcium binding protein A8 (calgranulin A); protein S100-A8; 60B8AG; CGLA; MRP8; P8; MRP-8; calgranulin A; calgranulin-A; cystic fibrosis antigen; calprotectin L1L subunit; urinary stone protein band A; leukocyte L1 complex light chain; migration inhibitory factor-related protein 8; S100 calcium-binding protein A8 (calgranulin A); MIF; NIF; CAGA; CFAG; L1Ag; CP-10; MA387;
GeneID	6279
mRNA Refseq	NM_002964
Protein Refseq	NP_002955
MIM	123885
UniProt ID	P05109
Chromosome Location	1q12-q22
Function	calcium ion binding; protein binding;

REFERENCES

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3. Sorg, C. (1992) Behring Inst. Mitt. 91, 126-137.

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