

S100A7 (Human) ELISA Kit

Cat. No.:DEIA4159

Pkg.Size:96T

Intended use

This kit is used for the quantitative measurement of human S100A7/Psoriasis in washing fluid from skin, nipple fluid, tissue culture medium and other biological samples probably including serum, plasma.

General Description

Human Psoriasis was first identified as an over-expressed secreted protein in psoriatic skin. Psoriasis also called S100A7, is distributed in the cytoplasm of keratinocytes in normal human epidermis and is present at the cell periphery in terminally differentiated keratinocytes. The peripheral distribution observed in differentiated cells may be important, since, under some conditions, S100A7 may be released from keratinocytes. Several S100 proteins are thought to be secreted. Indeed, S100A7 has been shown to function as a chemotactic agent and as a cytokine, and to attract CD4+ lymphocytes and neutrophils. Originally characterized as a marker of psoriasis, S100A7 overexpression is seen in many epidermal inflammatory diseases, including atopic dermatitis, mycosis fungoides, Darier's disease, and inflammatory lichen sclerosus atrophicus. The high level of expression in active psoriatic lesions has prompted investigators to suggest that S100A7 may have a chemotactic role in psoriasis. S100A7 expression is also increased in invasive skin cancers such as squamous cell carcinoma, and squamous carcinoma in situ, but not in basal cell carcinoma. It should be noted that 11-kD S100A7 protein purified from skin extract preferentially killed the gut bacterium Escherichia coli, but had little or no activity against Staphylococcus aureus or other bacteria. The activity could be inhibited by zinc, but not by other bivalent ions, suggesting that S100A7 kills E. coli by sequestration of zinc. Immunohistochemical analysis demonstrated intense expression of S100A7 in healthy skin, particularly in the face, scalp, and sebaceous glands. Real-time PCR and ELISA analysis of keratinocytes stimulated with E. coli culture supernatants revealed induced transcription of S100A7 and its secretion. Stimulation with IL-1 beta or, to a lesser extent, with TNF alpha also induced S100A7 transcription and secretion. These results indicated that S100A7 is key to the local innate defense against E. coli on body surfaces and kills the bacteria by sequestering essential transition metalions.

Principle Of The Test

This kit employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human S100A7/Psoriasis has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human S100A7/Psoriasis present is bound by the immobilized antibody. After washing away any unbound substances, an HRP conjugated polyclonal antibody specific for human S100A7/Psoriasis 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 S100A7/Psoriasis. A standard curve is constructed by plotting absorbance values versus human S100A7/Psoriasis 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-S100A4 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 20 mL of 1X buffer; use for sample dilution. Ready to use.
4. Human S100A7/Psoriasis Standard: One vial containing 360 ng of lyophilized recombinant S100A7/Psoriasis.
5. HRP conjugated Detection Antibody: One vial containing 12 mL of HRP (horseradish peroxidase) conjugated anti-S100A4 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 supplied ready to use, containing 20 mL of 1 N H₂SO₄.

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

Washing fluids from skin require neat.

Other biological samples require neat to a 5-fold dilution. e.g. 50 µL of sample + 200 µL of Dilution Buffer

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 S100A7/Psoriasis 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 S100A7/Psoriasis Standard with 1 mL of Dilution Buffer. The concentration of the Human S100A7/Psoriasis in vial should be 360 ng/mL, which is referred as a Master Standard of Human S100A7/Psoriasis.

Prepare Standard solutions as follows:

Use the Master Standard to produce a dilution series. Mix each tube thoroughly before the next transfer. The 90 ng/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 biological samples 1:5 with Dilution Buffer if necessary.

3. Pipette 100 µL of Human S100A7/Psoriasis Standards (Std1-Std7, Blank) and 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 S100A7/Psoriasin concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculation

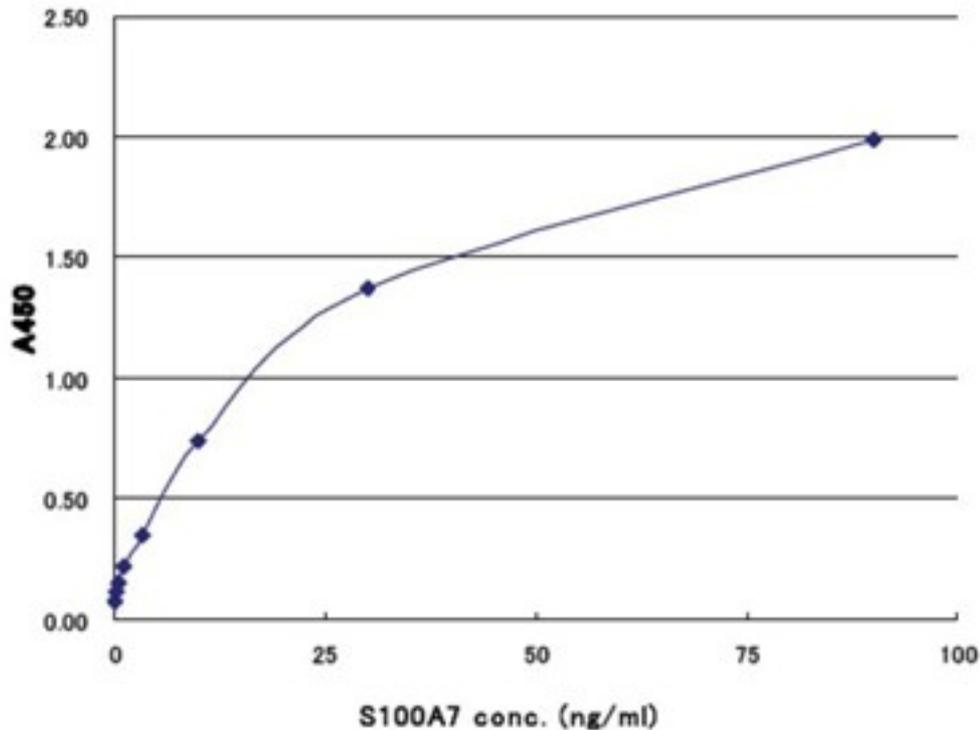
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. To determine the human S100A7/Psoriasin concentration of each sample, 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 S100A7/Psoriasin 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. log 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



Detection Range

Dilution factors need to be taken into consideration in calculating the human S100A7/Psoriasis concentration. Results exceeding human S100A7/Psoriasis level of 90 ng/ml should be repeated with diluted samples.

Sensitivity

The limit of detection (defined as such a concentration of human S100A /Psoriasis 7 giving absorbance higher than mean absorbance of blank plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3SD_{\text{blank}}$) is better than 0.12 ng/ml of sample.

Precautions

1. Allow all the components to come to room temperature before use.
2. 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.
3. Do not use kit components beyond the indicated kit expiration date.
4. Use only the microtiter wells provided with the kit.
5. Rinse all detergent residue from glassware.
6. Use deionized water of the highest quality.
7. Do not mix reagents from different kits.
8. The buffers and reagents used in this kit contain NaN_3 as preservatives. Care should be taken to avoid direct contact with these reagents.
9. Do not mouth pipette or ingest any of the reagents.
10. Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.

11. Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
12. Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide.
13. 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.
14. Human 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.
15. CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.

Analyte Gene Information

Gene Name	S100A7 S100 calcium binding protein A7 [Homo sapiens]
Official Symbol	S100A7
Synonyms	S100A7; S100 calcium binding protein A7; PSOR1, S100 calcium binding protein A7 (psoriasis 1) , S100 calcium binding protein A7 (psoriasis 1); protein S100-A7; S100A7c; psoriasis 1; S100 calcium-binding protein A7 (psoriasis 1); PSOR1;
GeneID	6278
mRNA Refseq	NM_002963
Protein Refseq	NP_002954
MIM	600353
UniProt ID	P31151
Chromosome Location	1q21
Pathway	Validated targets of C-MYC transcriptional repression, organism-specific biosystem;
Function	RAGE receptor binding; calcium ion binding; protein binding; zinc ion binding; zinc ion binding;

REFERENCES

1. Madsen P, Rasmussen HH & Leffers H et al. Molecular cloning, occurrence, and expression of a novel partially secreted protein "psoriasis" that is highly up-regulated in psoriatic skin. J Invest Dermatol (1991) 97: 701–712.
2. Broome AM, Ryan D & Eckert RL. S100 protein subcellular localization during epidermal differentiation and psoriasis. J Histochem Cytochem (2003) 51: 675–685.