IMMUNOLOGICAL SCIENCES Product Data Sheet

IK 4646 Human S100 calcium binding protein A12, S100A12 ELISA Kit

Size: 96T

Range: 10 ng/ml- 160 ng/ml

Sensitivity: 1 ng/ml

Application: For quantitative detection of S100A12 in human serum, plasma, cell culture supernatant or tissue homogenate.

Introduction: Protein S100-A12 is a protein that in humans is encoded by the S100A12 gene. The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100A12 is involved in the inflammatory response and is considered an important marker for many inflammatory diseases in humans. Li X et al found that the transcriptional activity and expression levels ofC/EBP and AP-1 could positively regulate each other. Furthermore, the promoter activity of the S100A12 gene was higher when C/EBP and AP-1 were cotransfected than when they were transfected individually. They concluded that the S100A12 gene was cooperatively and positively regulated by C/EBPand AP-1 in pigs. Malnutrition is highly prevalent and contributes to mortality in hemodialysis (HD) patients. Plasma S100A12 levels could play an important role in determining muscle mass and fat mass in HD patients.

Storage and Expiration: Store at 2-8C for 6 months.

Principle of the Assay: This kit was based on standard sandwichenzyme-linkedimmune-sorbentassay technology. The purified anti- S100A12antibody was pre-coated onto 96-well plates. And the HRP conjugated anti- S100A12 antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. TMB substrates (A & B) were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the S100A12 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of S100A12 can be calculated.

Kit components

1. One 96-well plate pre-coated with antihuman S100A12 antibody
2. Standard: 0.6ml (320 ng/ml)
3. Standard diluent buffer: 2 ml
4. Wash buffer (30X): 20 ml. Dilution: 1:30
5. Biotin conjugated anti- human S100A12 antibody (RTU): 1.5 ml
6. HRP-conjugated reagent: 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided

1. 37C incubator
2. Microplate reader (wavelength: 450nm)
3. Precise pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml of Eppendorf tubes
7. Absorbent filter papers
8. Plastic or glass container with volume of above 1L

Protocol

Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Use a serum separator tube and allow samples to coagulate at room temperature for 30 min, then, centrifuge at approximately 1000xg for 15 min to collect supernatant. If precipitation appeared, centrifuge again. Analyze immediately or aliquot and store at -20C or -80C.
- Plasma: Collect plasma using EDTA or sodium citrate as an anticoagulant, centrifuge at 1000xg for 15 min at 2-8C within 30 min of collection. If precipitation appeared, centrifuge again. Analyze immediately or aliquot and store at -20C or -80C.
- Cell culture supernatant: Remove particulates by centrifugation and analyze immediately or aliquot and store at -20C or -80C.
- Tissue samples: Cut samples and weight, add certain volume of PBS (pH7.4), rapidly frozen with liquid nitrogen. After melting, store samples at 2-8 C . Add certain volume of PBS (pH7.4), homogenize thoroughly, centrifuge at the speed of 2000-3000 r.p.m. for 20 min to collect supernatant.

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle.

2. NaN3 cannot be used as test sample preservative, since it is the inhibitor for HRP.
3. After collecting samples, analyze immediately or aliquot and store frozen at -20°C. Avoid repeated freeze-thaw cycles.

2**. Wash buffer**

Dilute concentrated Wash buffer (Kit Component 4) 30-fold (1:30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Dilution of Standard: Label 5 Eppendorf tubes with 160 ng/ml, 80 ng/ml, 40 ng/ml, 20 ng/ml, 10 ng/ml, respectively, add 100µl of Standard diluent buffer into each tube, then, add 100µl of Standard (Kit Component 2) to the 1st tube and mix thoroughly; transfer 100µl from the 1st tube to the 2nd tube and mix thoroughly; then transfer 100µl from the 2nd tube to the 3rd tube, then transfer 100µl from the 3rd tube to the 4th tube, then transfer 100µl from the 4th tube to the 5th tube, then aspirate 100µl from the 5th tube and discard.

Assay procedure

1. Equilibrate kit components for 15-30 min to room temperature (21-26C).
2. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. Add 50µl of diluted standards (160 ng/ml, 80 ng/ml, 40 ng/ml, 20 ng/ml, 10 ng/ml) into the standard wells. It is recommend to measure each standard in duplicate. Add 50µl of Standard diluent buffer (Kit Component 3) into the control (zero) well. Do not add sample, biotin conjugated antibody and HRP conjugated reagent into the control (zero) well.

3. For test sample wells, add 40µl of sample first, then, add 10µl of Biotin conjugated anti-human S100A12 antibody (Kit Component 5). Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37℃ for 30 min.
5. Remove the sealer, and wash plate using one of the following methods:
   Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers. Fill each well completely with Wash Buffer (1×) and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers. Repeat this procedure four more times for a total of FIVE washes.
   Automated Washing: Aspirate all wells, then wash plates FIVE times using Wash Buffer (1×). After the final wash, invert plate, and clap the plate on absorbent filter papers until no moisture remained. It is recommended that the washer be set for a soaking time of 10 seconds or shaking.
6. Add 50µl of HRP conjugated reagent (Kit Component 6) into each well (except control well).
7. Cover the plate with Plate sealer (Kit Component 10) and incubate at 37℃ for 30 min.
8. Remove the sealer, and wash the plate. (See Step 5)
9. Add 50µl of TMB chromogenic reagent A (Kit Component 8) into each well, and then, add 50µl of TMB chromogenic reagent B (Kit Component 9), vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds), and incubate in dark at 37°C for 15 min. The shades of blue can be seen in the wells.
10. Add 50µl of Stop solution (Kit Component 7) into each well and mix thoroughly. The color changes into yellow immediately.
11. Read the O.D. absorbance at 450nm in a microplate reader within 15 min after adding the stop solution.

    For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human S100A12 concentration of the samples can be interpolated from the standard curve.
    Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Precautions

1. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.
2. It is recommend to measure each standard and sample in duplicate.
3. Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.
4. Do not reuse pipette tips and tubes to avoid cross contamination.
5. Do not use the expired components and the components from different batches.
6. Store the TMB substrate A & B (Kit Component 8 & 9) in dark.
7. Prolong the incubation time if the hypochromasia obtained. Heat the water in the water bath during diluting if the crystalloid appeared in Wash buffer (Kit Component 4).
8. Do not remove microplate from the storage bag until needed, and the unused strips should be stored at 2-8℃ in their pouch or the provided Hermetic bag (Kit Component11).