EIA For Rat S100A9

Rat S100A9 Assay Kit

NOTE FOR USE

1. This kit is for research use only.
2. Users are recommended to read all instructions before use.
3. The assay procedure must be followed with indicated temperature and time.

KIT COMPONENTS

1.	Antibody Coated Plate: 96 microwells plate		1 plate
2.	S100A9 Standard 1 (3.75 ng/mL)	0.5mL	1 vial
3.	S100A9 Standard 2 (15 ng/mL)	0.5mL	1 vial
4.	S100A9 Standard 3 (60 ng/mL)	0.5mL	1 vial
5.	S100A9 Standard 4 (240 ng/mL)	0.5mL	1 vial
6.	Concentrated Diluent	50mL	1 vial
7.	Concentrated Washing Solution	50mL	1 vial
8.	Enzyme Conjugate	0.15mL	1 vial
9.	Color Developing Reagent A	11mL	1 vial
10.	Color Developing Reagent B	0.5mL	1 vial
11.	Stop Solution	11mL	1 vial

PRINCIPLE OF THE ASSAY

Assay principle of this kit is the solid phase enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies against rat S100A9 and rat S100A9 as a standard material. It is possible to measure the concentration of rat S100A9 in serum by this kit.

SAMPLE COLLECTION & PREPARATION

1. If samples are not analyzed immediately, they shall be kept at -20°C until assay.
2. Samples that have been repeated freeze-thaw cycles and/or hemolyzed sera shall not be used.
3. In the case of measuring the concentration of S100A9 in stool samples of rats, it needs to extract S100A9 from them. Please see "METHOD FOR EXTRACTING S100A9 FROM STOOL SAMPLES".
4. All kit components and samples are warmed up to room temperature (18 to 27°C) before use.

ASSAY PROCEDURE

A. Preparation of Reagents

1. Prepared Diluent
   Dilute Concentrated Diluent three fold with purified water.
2. Enzyme Conjugate Solution
   Mix Enzyme Conjugate and Prepared Diluent at a ratio of one to one hundred.
   This solution can be used for up to 28 days if stored at -20°C.
3. Substrate Mixture
   Mix Color Developing Reagent A and Color Developing Reagent B at a ratio of one hundred to one.
   Note: ***This solution should always be prepared just before use***.
4. Washing Solution
   Dilute Concentrated Washing Solution five fold with purified water.
   This solution can be used for up to 28 days if stored at 2 - 8°C.

B. Additionally Material Required

-Micropipettes (10, 100, 300 µL) with disposable plastic tip
-Vibratory mixer
-Microplate reader
-Plastic test tube (avoid to use glass test tube)
-Incubator
-Aspirator for microplate or Microplate Washer
-Purified water

C. Preparation of Sample

Sera and extract solution of stool samples of rats are diluted 10-fold or more fold with Diluent. If the S100A9 level of the sample exceed measuring range, dilute with Diluent to obtain a value within the range.

D. Standard Procedure for the Assay

Samples should be determined in duplicate. Make a work sheet with Prepared Standard for standard curve and diluted samples as shown in Fig.1. Standard curve should be drawn individually for each assay.

	1 2	3 4	5 6	7 8	9 10	11 12
A	S100A9 Standard 1 (3.75 ng/mL)	Sample 5				
B	S100A9 Standard 2 (15 ng/mL)					
C	S100A9 Standard 3 (60 ng/mL)					
D	S100A9 Standard 4 (240 ng/mL)					
E	Sample 1					
F	Sample 2					
G	Sample 3					
H	Sample 4					

Fig.1 Example of work sheet

1) Add 100µL of S100A9 Standard 1-4 and diluted samples to each well.
2) Incubate the plate at 20 - 30 ºC for 2 hours.
3) Remove mixture from each well. Add 300µL of Washing Solution to each well. Remove Washing Solution from each well. Repeat the above steps twice. Turn the plate upside down on a paper towel. Then, remove any residual liquid by tapping the plate on the blotting paper towel.
   Note: ***Take care not to dry the well.***
4) Add 100µL of Enzyme Conjugate Solution to each well.
5) Incubate the plate at 20 - 30 ºC for 1 hour.
6) Repeat step 3.
7) Add 100µL of Substrate Mixture to each well.
8) Incubate the plate at 20 - 30 ºC for 20 min.
9) Add 100µL of Stop Solution to each well.
10) Measure the absorbance of each well at 450 nm with Microplate reader.

E. Calculation of Results

Calculate the mean value of the absorbance for each set of S100A9 Standard 1 -4 and sample. The values (linear scale, y-axis) are plotted against the corresponding concentration of S100A9 Standard (logarithmic scale, x-axis). Draw a best-fit line through the points. S100A9 concentration of the samples can be calculated from the standard curve. Multiply dilution factor to the concentrations.

<u>METHOD FOR EXTRACTING S100A9 FROM STOOL SAMPLES</u>

(1) Preparation of a diluent for extraction
A diluent for extraction was prepared by adding Triton X-100 to Prepared Diluent at a final concentration of 0.5% (w/v).

(2) Equipment
Pestle, tube, etc.

(3) Extraction
1) Weigh the blank tube.
2) Collect about 200 mg of feces into the tube and weigh it.
3) Calculate the weight of the feces subtracting result of 1) from result of 2).
4) Add 500 µL of diluent for extraction into a fecal tube.
5) Stand the tube to stand for 15 minutes.
6) The mixtures were homogenized using a pestle.
7) Add 500 µL of diluent for extraction to each tube.

8) Centrifuge all tubes at 12,000 g for five minutes at 4 ºC. Collect the supernatant into a new tube.
9) Dilute the supernatant ten-fold or more with a diluent for extraction.
10) Determine S100A9 level in the supernatant using standard procedures to calculate the amount of S100A9 in 1 mg of feces.

PRECAUTION FOR USE OR HANDLING

1. The samples from rat should be handled with care, as all materials of animal are potentially hazardous.
2. Stop Solution contains sulfuric acid and should be handled with care.
3. Should reagents get into your eyes or mouth, immediately rinse them with water. Take medical advice if necessary.
4. Prepared reagents should be stored under the condition described on this instruction manual.
5. Reagents from different kit lot numbers should not be combined or interchanged.
6. Any expired components should not be use.

STORAGE AND STABILITY

Store all components at 2 to 8 °C. This kit is stable for 24 months under this condition from manufacturing date. The expiry date of kit is printed on the label of outer box.

BIBLIOGRAPHY

1. Murayama H. et al. The 42nd Annual Meeting of The Japanese Society of Toxicology
2. Sekiya S et al. (2016). J Immunol Methods. 439. 44–49.