Chondrex, Inc.

Mouse S100A8 Detection ELISA Kit

Catalog # 6054

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse S100A8
FORMAT:	96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5 hours
STANDARD RANGE:	100 – 1.6 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Mouse serum, culture medium and biological fluids
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (2.8-4.4%) /Inter-Assay (2.6-9.2%)/ Spiking Test (96-109%)
NOTES:	N/A

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INTRODUCTION

During an inflammatory response, neutrophils and monocytes are among the first innate immune cells to migrate to the site of inflammation. These cells actively or passively release the heterodimeric protein S100A8/A9 known as calgranulin A or MRP-8. The S100A8/A9 exerts its effects by binding to the pattern recognition receptors such as Toll-like receptor 4 and the Receptor for Advanced Glycation Endproducts, leading to activating inflammatory signaling cascades (1).

S100A8 is composed of 93 amino acids (AAs) with a molecular weight (MW) of 10.8 kDa, whereas S100A9 consists of 113 AAs (MW 13.2 kDa). A truncated isoform of S100A9 containing 110 AAs (MW 12.7 kDa) has also been identified. Although both S100A8 and S100A9 are capable of forming homodimers in vitro, the proteins preferentially assemble into a stable heterodimer in serum (1).

S100A8/A9 levels in plasma have been widely used as a biomarker for various inflammatory disorders (1). Especially, serum S100A8/A9 levels correlate strongly with disease activity in mouse inflammatory disease models, including collagen-induced arthritis (2), DSS-induced colitis (3), sepsis (4) and skin inflammation (5). Furthermore, a significant elevated S100A8/A9 expression has been found in mouse tumor models, such as breast (6), lung (7), and gastric cancers (8).

To investigate the role of S100A8/A9 in mouse inflammatory disease models, Chondrex, Inc. offers a mouse S100A8 detection ELISA kit (cat# 6054) for assaying the protein in biological samples such as serum, plasma, or culture medium, as well as reagents for inducing experimental disease models. Additionally, we provide a mouse MPO detection ELISA kit (Cat to evaluate Neutrophil Extracellular Trap (NET) formation, which is related to neutrophil activation research.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Recombinant mouse S100A8 standard (60541)	1 vial	100 ng, lyophilized	-20°C
Anti-mouse S100A8 Capture Antibody (60542)	1 vial	100 µl	-20°C
Anti-mouse S100A8 Detection Antibody (60543)	1 vial	100 µl	-20°C
Solution A - Coating Buffer (9052)	1 bottle	10 ml	-20°C
Solution C - Sample/Standard Dilution Buffer (30106)	1 bottle	50 ml	-20°C
Solution D – Blocking/Detection Antibody/ Streptavidin Peroxidase Dilution Buffer (9055)	2 bottles	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
ELISA Plate	1 each	96-well (8-well strips x 12)	-20°C

ASSAY OUTLINE

Add 100 µl of diluted Capture Antibody Solution into wells.

↓ Incubate at 4°C overnight.
Wash plate.

Add 100 µl of blocking buffer into wells.

↓ Incubate at room temperature for 1 hour
Wash plate.

Add 100 µl of diluted standards or samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted Detection Antibody into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase into wells

↓ Incubate at room temperature for 30 minutes.
Wash plate.

Add 100 µl of TMB solution into wells

↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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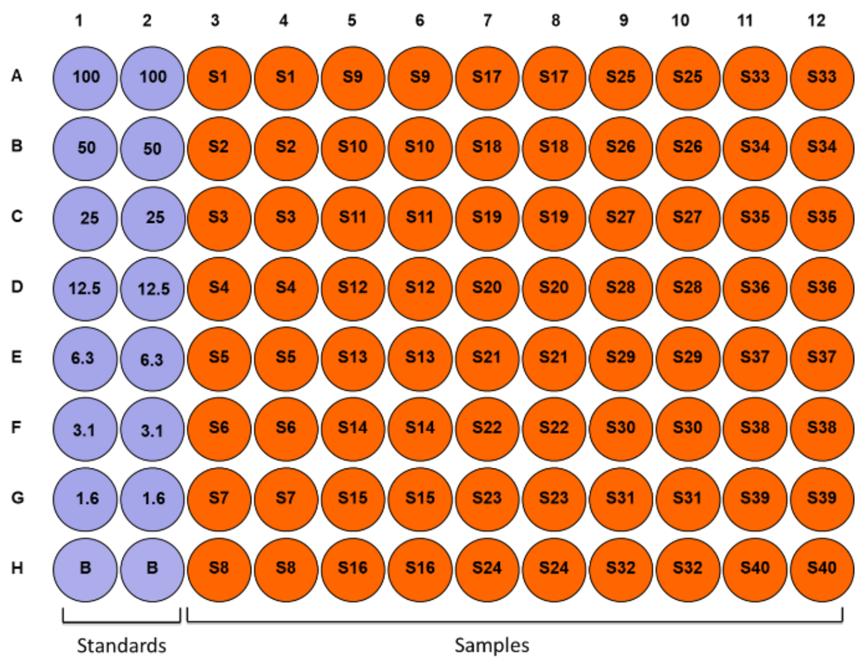
NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

PLATE MAPPING



ASSAY PROCEDURE

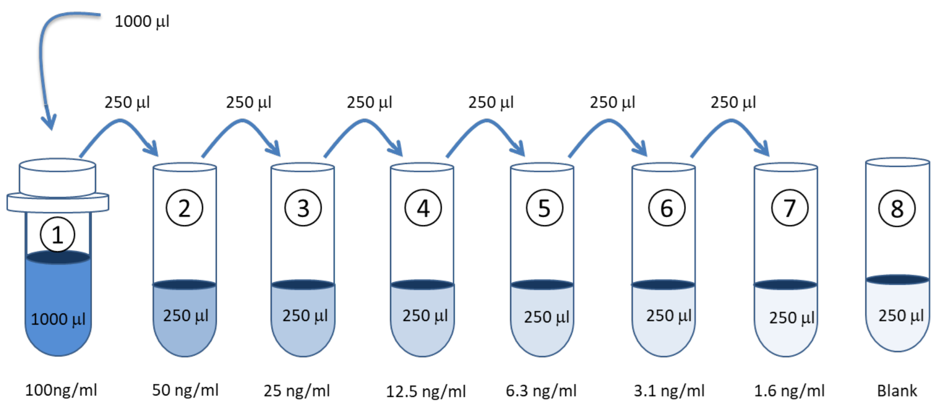
1. **Add Capture Antibody**: Dilute one vial of Capture Antibody with 10 ml of Coating Buffer (Solution A). Alternatively, dilute according to the table below. Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight. Any remaining Capture Antibody Stock Solution can be stored at -20°C for future use.

Strip #	Capture Antibody (µl)	Solution A (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

3. **Add Blocking Buffer**: Add 100 µl of Blocking/Detection Antibody/ Streptavidin Peroxidase Dilution Buffer (Solution D) to each well and incubate at room temperature for 1 hour.

4. **Prepare Standard Dilutions**: The recommended standard range is 1.6 - 100 ng/ml. Dissolve one vial of Standard (1000 ng/vial) in 1 ml of Sample/Standard Dilution Buffer (Solution C) and keep it as a standard stock. Then serially dilute it with Solution C. For example, mix 250 µl of stock solution with an equal volume of Solution C to make the second stock solution, and then repeat it five more times. The remaining stock solution can be stored at -20°C for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



5. **Prepare Sample Dilutions**: Centrifuge samples at 10,000 rpm at 4°C for 3 minutes to remove insoluble materials and lipids and use the supernatant as samples. Dilute samples at least 1:1 with Solution C depending on the estimated S100A8 level in the samples. Two or three different sample dilutions are recommended if the S100A8 levels in the samples are unknown.

6. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add S100A8 Detection Antibody**: Dilute one vial of Detection Antibody in 10 ml Blocking/Detection Antibody/ Streptavidin Peroxidase Dilution buffer (Solution D). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution D (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

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10. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

12. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

13. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

14. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

15. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATING RESULTS

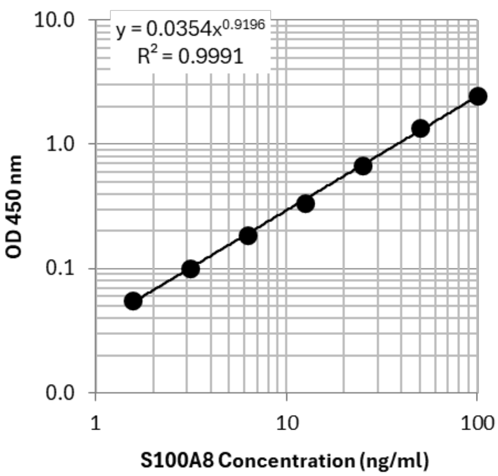
1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the ng/ml of standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve where the standard range is 1.6 to 100 ng/ml.

4. The ng/ml of S100A8 in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the S100A8 concentration (ng/ml) in the original test samples.

Figure 1 - A Typical Standard Curve for the S100A8 Detection ELISA Kit



VALIDATION DATA

Table 1 - Reproducibility Data for the S100A8 Detection ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	4.4	2.8	3.9
Inter-Assay CV (%)	9.2	5.1	2.6
Spike Test* (%)	97	109	96

*Known amounts of mouse S100A8 were added to samples and then diluted with Sample/Standard Dilution Buffer to assay S100A8 by ELISA.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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