Feline Serum Amyloid A Detection Kit

Catalog # 6028

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Acute phase proteins (APPs) are blood proteins that are an integral part of acute phase responses (APR). The APR is a part of the innate host defense system which is triggered by tissue damage and inflammation caused by infectious, immunologic, or neoplastic agents. One of the well-characterized APPs, serum amyloid A (SAA) is a 11.4 - 12.5 kDa protein consisting of 104 - 112 amino acids in different species. The protein is well-conserved in the evolution of eutherian mammals, indicating an important role in biological functions. The N-terminus of the molecule is hydrophobic and probably responsible for its lipid-binding properties which allows SAA to form complexes with high-density lipoproteins as well as apoproteins (1). In inflamed tissues, macrophages serve as a major source of SAA.

In fact, during APR, SAA levels in plasma increase 1000-fold in human and other species, suggesting it may be an important indicator of disease status. Elevated SAA levels are also seen in rheumatoid arthritis, atherosclerosis, Crohn's disease, and Type 2 diabetes. These findings suggest that SAA may play an active and/or a protective role in inflammatory disorders (2, 3).

In veterinary medicine, Tamamoto *et.al.* indicated that measuring feline SAA concentration, in addition to white blood cell counts, would be clinically valuable as a routine test to detect inflammation (4). Furthermore, SAA levels work as a significant and independent prognostic marker in cats with various diseases such as neoplastic diseases and inflammatory diseases (5).

Chondrex, Inc provides a feline serum SAA ELISA detection kit (Catalog # 6028) which can be used to detect and monitor feline inflammation, as well as in studies investigating inflammation. Please contact Chondrex, Inc. at support@chondrex.com for more information.

KIT COMPONENTS

Item	Quantity	Amount	Storage
SAA Standard (60281)	1 vial	2000 ng, lyophilized	-20°C
Detection Antibody (60283)	1 vial	100 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB (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
Capture Antibody Coated 96-Well ELISA Plate	1 each	8-well strips x 12	-20°C

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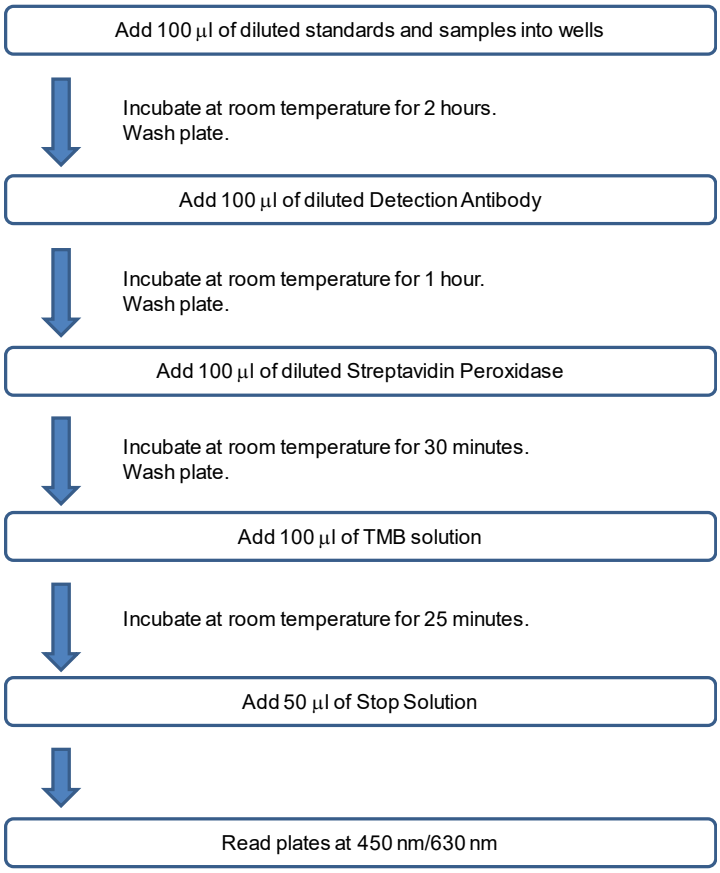
ASSAY OUTLINE



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: Partially used reagents may be kept at –20°C.

Note 4: 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 dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

Note 6: 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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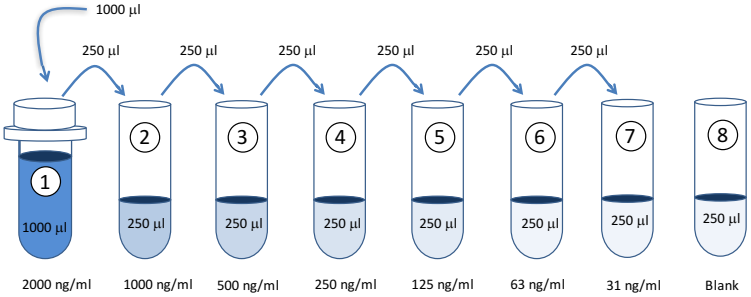
ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 31 - 2000 ng/ml. Dissolve one vial of SAA standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 2000 ng/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (2000 ng/ml) with an equal volume of Solution B to make a 1000 ng/ml solution, and then repeat it five more times for 500, 250, 125, 63, and 31 ng/ml solutions. The remaining 2000 ng/ml standard stock may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



2. **Prepare Samples**:

   Serum: Clot blood samples by incubating samples at room temperature for 2 hours

   Plasma: Collect blood samples with the use of anticoagulants such as heparin. Then, collect plasma by centrifuging samples at 10,000 rpm for 5 minutes within 30 minutes of blood collection. When not in use, store the plasma supernatant samples at -20°C; avoid repeat freeze-thaw cycles.

   Note : Using lipemic (cloudy) samples may affect assay results. The stored samples must be centrifuged at 10,000 rpm for an additional 5 minutes before the assay.

   Dilution: Dilute samples at least 1:1 with Solution B depending on the estimated SAA level in the samples. Two to three different sample dilutions are recommended if the SAA levels in the samples are unknown.

   Note: Samples must be diluted with Solution B to maintain optimal assay conditions.

3. **Add Standards and Samples**: Add 100 µl of Solution B (blank), standards, and samples to designated wells in duplicate according to the layout in Figure 1. Incubate at room temperature for 2 hours.

Figure 1 - A Standard Assay Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	2000	2000	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	1000	1000	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	500	500	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	250	250	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	125	125	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	63	63	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	31	31	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards Samples

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4. **Dilute Wash Buffer**: Dilute 50 ml of Wash Buffer, 20X 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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Detection Antibody Solution**: Prepare detection antibody solution with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) as shown in the following table.

Strip #	Detection Antibody (µl)	Solution B (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

Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

6. **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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.8
4	17	3.4
6	25	5.0
8	33	6.6
10	41	8.2
12	50	10.0

Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **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.

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

Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

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

11. **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.

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CALCULATION OF RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.
2. Subtract the "blank" (B) values from the averaged OD values in step 1.
3. Plot the OD values of standards against the concentration of SAA (ng/ml). Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is 31 - 2000 ng/ml.
4. The ng/ml of SAA in test samples can be calculated using regression analysis.

Figure 2 - A typical standard curve for SAA assay

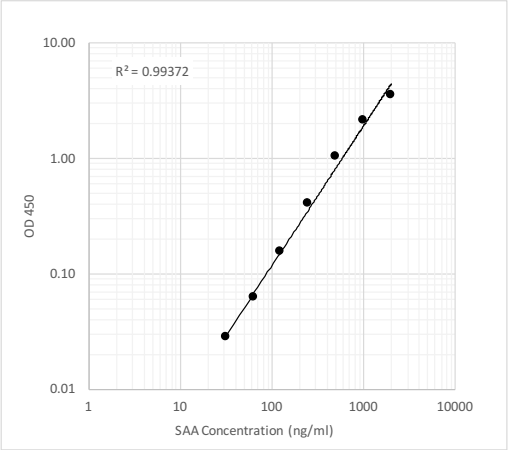

Table 1 - Reproducibility for SAA ELISA Kit

Test	63 ng/ml	250 ng/ml	1000 ng/ml
Inter-Assay CV (%)	5.1	6.1	9.6
Intra-Assay CV (%)	5.7	6.6	8.1
Spike Test*	109%	104%	109%

* Known amounts of SAA were added to feline serum and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

REFERENCES

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3. K. Sasaki, Z. Ma, T. Khatlani, M. Okuda, H. Inokuma, T. Onishi, *et al.*, Evaluation of feline serum amyloid A (SAA) as an inflammatory marker. *J Vet Med Sci* **65**, 545-8 (2003).
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