Dog Serum Amyloid A ELISA Assay Kit

Catalog Number: CSA49-K01 (1 x 96 Wells)
For Research Use Only. Not for diagnostic purposes.

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INTENDED USE

The Canine (Dog) Serum Amyloid A (SAA) ELISA Assay Kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring SAA in biological fluid of Dogs.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

ASSAY PRINCIPLE

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the SAA present in samples reacts with the anti-SAA antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-SAA antibodies conjugated with horseradish peroxidase (HRP.) are added. These enzyme-labeled antibodies form complexes with the previously bound SAA. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of SAA in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of SAA in the test sample. The quantity of SAA in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

REAGENTS PROVIDED AND REAGENT PREPARATION

Store all other reagents at 2 to 8°C. Use only reagents supplied with. Do not interchange reagents with different lot numbers. Expiration dates and lot numbers are printed on the labels.

1. Microplate

Contents:	Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Dog SAA.
Format:	Ready to use
Storage:	2 – 8ºC
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

2. Enzyme Conjugated Detection Antibody

Contents:	One vial containing affinity purified anti-Dog SAA antibody conjugated with horseradish peroxidase in a stabilizing buffer.
Format:	Ready to Use
Volume:	150 µL
Storage:	2 - 8ºC
Stability:	The working solution should be diluted immediately prior to use. The 100X conjugate is stable until the expiration date.
Preparation:	Dilute 1/100 immediately prior to use.

3. Dog SAA Calibrator

Contents:	One vial of Calibrator
Format:	Ready to Use
Storage:	2 - 8ºC
Preparation:	Prepare according to the lot specific CoA
Stability:	Store at 4ºC or Frozen prior to use. Prepare Immediately prior to use. The reconstituted calibrator should be aliquoted out and frozen.

4. Diluent Concentrate

Contents:	One bottle containing a 5X concentrated diluent running buffer
Format:	Concentrated, Needs preparation
Storage:	2 - 8ºC
Preparation:	Dilute 1/5 with distilled or deionized water
Stability:	Diluent concentrate is stable until the expiration. The 1X working solution is table for at least one week from date of preparation.

5. Wash Solution Concentrate

Contents:	One bottle containing 20X Wash Solution
Format:	Concentrated, Needs preparation
Volume:	50 mL / bottle
Storage:	2 - 8ºC
Preparation:	Dilute 1/20 to make a 1X working solution
Stability:	The 1X working solution is stable for at least one week from the date of preparation. The 20x concentrate is stable until the expiration date.

6. Chromogen-Substrate Solution

Contents:	One bottle containing 3,3' ,5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at PH 3.3.
Format:	Ready to Use
Volume:	12mL / bottle
Storage:	2 - 8ºC in the dark
Stability:	Protect from light. Stable until expiration

7. Stop Solution

Contents:	One bottle containing 0.3M sulfuric acid
Format:	Ready to Use
Volume:	12 mL / bottle
Storage:	2 - 8ºC
Stability:	Stable until expiration date

STORAGE AND STABILITY

- When stored at 2-8C, unopened reagents will retain activity until the expiration date. Do not use reagents beyond this date
- Use only reagents supplied with this kit. Do not interchange reagents with different lot numbers
- Opened reagents must be stored at 2-8C
- Microtiter wells must be stored at 2-8C. Once foil bag has been opened, care should be taken to reseal tightly.
- Expiration dates and lot numbers are printed on the labels

MATERIALS NEEDED BUT NOT SUPPLIED

1. Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
2. Test Tubes
3. Squirt bottle or Microtitre washer/aspirator
4. Distilled or Deionized water
5. Microtitre plate reader
6. Assorted glassware for the preparation of reagents and buffer solutions
7. Centrifuge for sample collection
8. Anticoagulant for plasma collection

SPEICMEN COLLECTION AND HANDLING

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions when handling and disposing.

If blood samples are clotted, grossly hemolyzed, lipemic, or the integrity of the sample is of concern, make a note and interpret results with caution.

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

- **Serum samples** - Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Remove serum and assay immediately or aliquot and store samples at -80°C (preferably) or -20°C. Avoid repeated freeze-thaw cycles.
- **Plasma samples** - Blood should be collected into a container with an anticoagulant and then centrifuged. Assay immediately or aliquot and store samples at -80° C (preferably) or -20° C. Avoid repeated freeze-thaw cycles.
- **Urine samples** - Collect mid-stream using sterile or clean urine collector. Centrifuge to remove cell debris. Assay immediately or aliquot and store samples at -80° C (preferably} or -20°C. Avoid repeated freeze-thaw cycles.
- **Known interfering substances** - Azide and thimerosal at concentrations higher than 0.1 % inhibits the enzyme reaction.

DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the

standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

- **Serum samples** - Recommended starting dilution is 1/200. To prepare a 1/200 dilution of a sample, transfer 2 µL of sample to 398 µL of 1 X diluent. This gives you a 1/200 dilution. Mix thoroughly.
- **Plasma samples** - Recommended starting dilution is 1/10,000. To prepare a 1/10,000 dilution of a sample, transfer 5 µL of sample to 495 µL of 1X diluent. This gives you a 1/100 dilution. Next, dilute the 1/100 by transferring 5 µL into 495 µL of 1X diluent. This gives you a 1/10,000 dilution. Mix thoroughly each stage.

REAGENT PREPARATION

- **Bring all reagents to room temperature (16°C to 25°C) before use.**
- **Diluent Concentrate** - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with dis-tilled or deionized water (1 part buffer concentrate, 4 parts dH2O).
- **Wash Solution Concentrate** - The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.
- **Enzyme-Antibody Conjugate** - Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.
- **Pre-coated ELISA Micro Plate** - Ready to use as supplied. Unseal foil pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.
- **Dog SAA Calibrator** - Prepare according to the lot specific Certificate of Analysis.

ASSAY PROCEDURE

1. All samples and standards should be assayed in duplicates.
2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

   Pipette 100µL of

   Standard 0 - (0.0 ng/ml) in duplicate
   Standard 1 - (6.25 ng/ml) in duplicate
   Standard 2 - (12.50 ng/ml) in duplicate
   Standard 3 - (25 ng/ml) in duplicate
   Standard 4 - (50 ng/ml) in duplicate
   Standard 5 - (100 ng/ml) in duplicate
   Standard 6 - (200 ng/ml) in duplicate

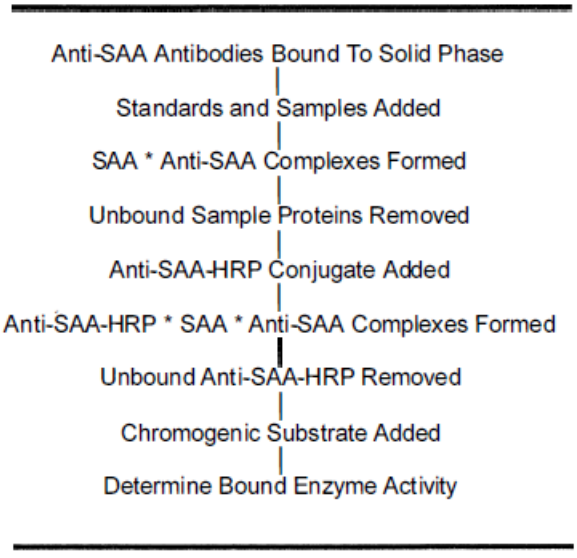
3. Pipette 100 µL of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.
7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for thirty (30 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 516:
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

CALCULATION OF RESULTS

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the SAA concentration in original samples.

Condensed procedure



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For further information about this kit, its application, or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.