KAMIYA BIOMEDICAL COMPANY

Horse Serum Amyloid A ELISA

For the quantitative determination of Serum Amyloid A (SAA) in horse biological fluid

Cat. No. KT-660

For research use only.

PRODUCT INFORMATION

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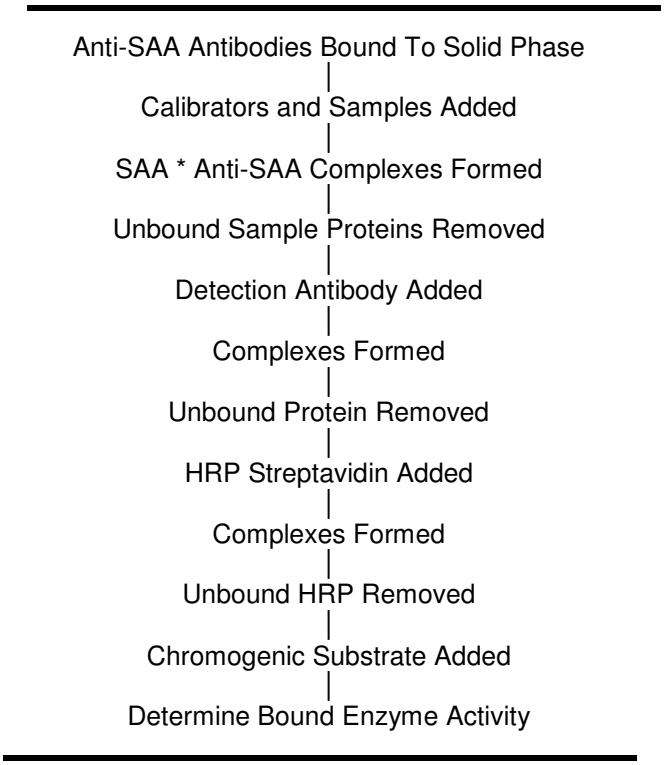
INTENDED USE
The Horse SAA ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of SAA in horse biological fluid. For research use only.

PRINCIPLE
The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the SAA present in the sample reacts with the anti-SAA antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, the Detection Antibody, biotin conjugated anti-SAA, is added and complexes are formed. Following a wash step, the horseradish peroxidase (HRP) conjugated Streptavidin is added and complexes are formed. After another washing step, the complexes are assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme is proportional to 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 calibration curve constructed from the calibrators, and corrected for sample dilution.

Figure 1.



COMPONENTS
1. Diluent Concentrate
   One bottle containing 60 mL of a 1X concentrated diluent running buffer.

2. Wash Solution Concentrate
   One bottle containing 50 mL of a 20X concentrated wash solution.

3. Detection Antibody 100X
   One vial containing 150 µL of affinity purified anti-Horse SAA antibody conjugated with biotin in a stabilizing buffer.

4. HRP-Streptavidin 100X
   One vial containing 150 µL of Horseradish Peroxidase conjugated streptavidin in a stabilizing buffer.

5. TMB Substrate Solution
   One vial containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.

6. Stop Solution
   One vial containing 12 mL of 0.3 M sulfuric acid.
   WARNING: Avoid contact with skin.

7. Microtiter Plate
   Twelve removable eight-well micro strips in well holder frame. Wells are coated with affinity-purified anti-horse SAA.

8. Horse SAA Calibrator
   One vial containing a lyophilized Horse SAA Calibrator.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microplate washer/aspirator
- Distilled or de-ionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Orbital Shaker

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
5. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
6. Other precautions:
   - Do not interchange kit components from different lots.
   - Do not use kit components beyond the expiration date.
   - Protect reagents from direct sunlight.
   - Do not pipette by mouth.
   - Do not eat, drink, smoke or apply cosmetics where reagents are used.
   - Avoid all contact with the reagents by using gloves.
   - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION

1. Diluent Concentrate
   Ready to use as supplied.

2. Wash Solution Concentrate
   The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Detection Antibody 100X
   Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL detection antibody to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. HRP-Streptavidin 100X

Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL HRP-Streptavidin to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

5. TMB Substrate Solution
   Ready to use as supplied.

6. Stop Solution
   Ready to use as supplied.

7. Microtiter Plate
   Ready to use as supplied. Unseal Microtiter 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.

8. Horse SAA Calibrator
   Add 1.0 mL of distilled or de-ionized water to the Horse SAA calibrator and mix gently until dissolved. The calibrator is now at a concentration of 3.384 µg/mL **(the reconstituted calibrator should be aliquoted and frozen if future use is intended). Horse SAA calibrators need to be prepared immediately prior to use (see chart below).** Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator Volume added to 1X Diluent →	Volume of 1X Diluent
6	72	20 µL Horse SAA Calibrator	920 µL
5	36	0.3 mL Calibrator 6	0.3 mL
4	18	0.3 mL Calibrator 5	0.3 mL
3	9	0.3 mL Calibrator 4	0.3 mL
2	4.5	0.3 mL Calibrator 3	0.3 mL
1	2.25	0.3 mL Calibrator 2	0.3 mL
0	0		0.6 mL

STORAGE AND STABILITY

1. Complete Kit
   The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long term storage recommendations below for the Horse SAA Calibrator.**

2. Diluent
   The 1X Diluent Concentrate is stable until the expiration date and should be stored at 4°C.

3. Wash Solution
   The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (RT, 16-25°C) or at 4°C.

4. Detection Antibody 100X
   Undiluted Biotin conjugated anti-SAA should be stored at 4°C and **diluted immediately prior to use**.

5. HRP-Streptavidin 100X
   Undiluted horseradish peroxidase conjugated streptavidin should be stored at 4°C and **diluted immediately prior to use**.

6. TMB Substrate Solution
   The TMB Substrate Solution should be stored at 4°C and is stable until the expiration date.

7. Stop Solution
   The Stop Solution should be stored at 4°C and is stable until the expiration date.

8. Microtiter Plate
   Anti-horse SAA coated wells are stable until the expiration date and should be stored at 4°C in the sealed foil pouch with a desiccant pack.

9. Horse SAA Calibrator
   The lyophilized Horse SAA calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (avoid multiple freeze-thaw cycles). The working calibrator solutions should be prepared immediately prior to use and are stable for up to 8 hours.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freezing/thawing.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

ASSAY PROTOCOL

Dilution of Samples

The assay for quantification of SAA in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1:200 is appropriate for most serum/plasma samples. For absolute quantification of samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required. **If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.**

To prepare a 1:200 dilution of sample, transfer 2 µL of sample to 398 µL of 1X Diluent. This gives you a 1:200 dilution. Mix thoroughly.

Procedure

1. **Bring all reagents to RT before use.**

2. Pipette 100 µL of
   Calibrator 0 (0.0 ng/mL) in duplicate
   Calibrator 1 (2.25 ng/mL) in duplicate
   Calibrator 2 (4.5 ng/mL) in duplicate
   Calibrator 3 (9 ng/mL) in duplicate
   Calibrator 4 (18 ng/mL) in duplicate
   Calibrator 5 (36 ng/mL) in duplicate
   Calibrator 6 (72 ng/mL) in duplicate

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

4. Incubate the Microtiter Plate while shaking on an orbital shaker at 22°C (RT) 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 and 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 4 washes.

7. Pipette 100 µL of appropriately diluted detection antibody to each well. Incubate while shaking on an orbital shaker at 22°C (RT) for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 µL of appropriately diluted HRP-streptavidin to each well. Incubate while shaking on an orbital shaker at 22°C (RT) for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

10. Wash and blot the wells as described in Steps 5 and 6.

11. Pipette 100 µL of TMB Substrate Solution into each well.

12. Incubate in the dark while shaking on an orbital shaker at RT for precisely ten (10) minutes.

13. After ten (10) minutes, add 100 µL of Stop Solution to each well.

14. Determine the absorbance at 450 nm of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from calibration curve. Correct for sample dilution factor to arrive at SAA concentration in original sample.

QUALITY CONTROL

In accord with good laboratory practice, the assays for specific SAA require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature.

FOR RESEARCH USE ONLY

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