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ab274407 – Bovine SAA ELISA Kit

For the determination of SAA in bovine biological samples.

View ab274407
Bovine SAA ELISA Kit datasheet:
www.abcam.com/ab274407
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This product is for research use only and is not intended for diagnostic use.

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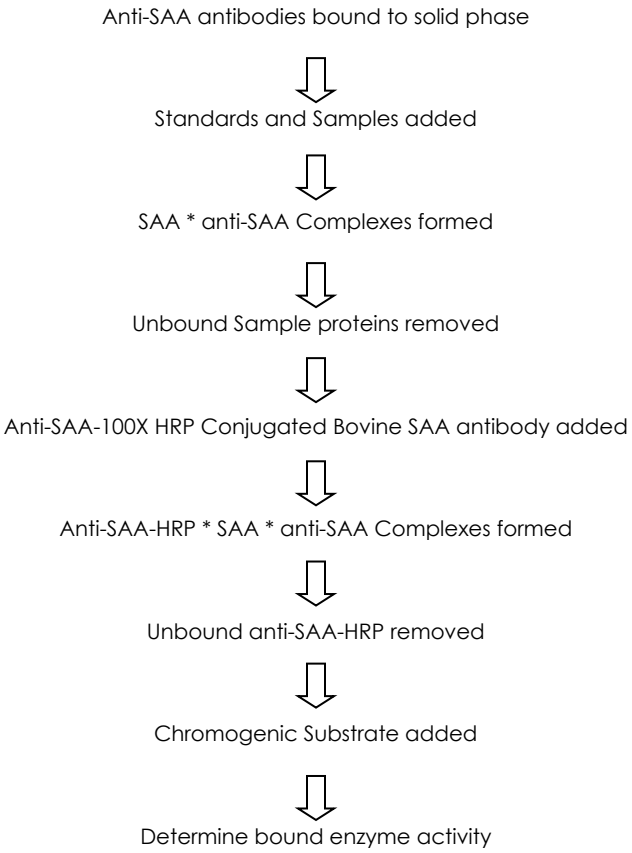
1. Overview

The Bovine SAA ELISA kit (ab274407) is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring SAA in bovine biological samples.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. Storage and Stability

- Store the components kit at +4-8°C in the dark immediately upon receipt.
- Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
- Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. Limitations

- This assay will perform as described only when the assay procedure is carefully followed and with adherence to good laboratory practice.
- Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, accuracy of reagent and sample pipetting, washing technique, incubation time and/or temperature.
- Do not mix or substitute reagents with those from other lots or sources.

6. Materials Supplied

Item	Quantity	Storage Condition
Antibody coated plate	96 wells	4°C
Bovine SAA Bovine SAA Calibrator (Lyophilized)	1 vial	4°C
5X Diluent Buffer	50 mL	4°C
20X Wash Buffer	50 mL	4°C
100X HRP Conjugated Bovine SAA antibody	1 vial	4°C (Store in dark)
Chromogen Substrate Solution	12 mL	4°C (Store in dark)
Stop Solution	12 mL	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtitre washer/aspirator
- Distilled or Deionized H_2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

8. Technical Hints

- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Completely aspirate all solutions and buffers during wash steps. When preparing your standards, it is critical to briefly spin down the vial first. The powder may adhere to the cape and not be included in the standard solution resulting in an incorrect concentration. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is an effective technique for thorough mixing of the standard without using excessive mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- The standard should be used immediately after reconstitution, or frozen for later use.
- Keep the standard dilutions on ice during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.

9.1 1X Diluent Solution

The diluent solution is supplied as 5X Diluent Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH_2O). The 1X Diluent Solution is stable for at least one week from the date of preparation and should be stored at +4-8°C.

9.2 1X Wash Buffer

The wash solution is supplied as 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). 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. The 1X Wash Buffer is stable for at least one week from the date of preparation and can be stored at +4-8°C.

9.3 HRP-Conjugate

Calculate the required amount of 1X Enzyme-Antibody 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 (1/100 dilution). Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

9.4 **Antibody coated 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.

9.5 **Bovine SAA Calibrator (Lyophilized)**

The calibrator is provided at the concentration stated on the vial. Reconstitute the Bovine SAA Calibrator with distilled or de-ionized water as specified on the vial and mix gently until dissolved. The amount of calibrator is shown on the vial, and after reconstitution will have a concentration of X µg/mL, where X is the amount on the vial (the reconstituted calibrator should be aliquoted and stored frozen if future use is intended). Prepare according to section 12.

9.6 **Chromogen Substrate and Stop Solution:**

Ready to use as supplied.

10. Sample Collection and Storage

- 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.
- Known interfering substances - Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

10.1 **Serum:** 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.

10.2 **Plasma:** 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.

10.3 **Urine:** 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.

11. Sample Preparation

General Sample information:

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.

11.1 **Serum**: Recommended starting dilution is 1/500. To prepare a 1/500 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 1/00 mix by transferring 120 µL into 480 of 1X diluent. Mix thoroughly.

11.2 **Plasma:** Recommended starting dilution is 1/500. To prepare a 1/500 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 1/00 mix by transferring 120 µL into 480 of 1X diluent. Mix thoroughly.

12. Assay Procedure

– We recommend that you assay all standards, controls and samples in duplicate.

12.1 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 100 µL of the below standards in duplicate:

Standard	Concentration (ng/mL)
0	0.0
1	26.34
2	39.51
3	59.26
4	88.89
5	133.33
6	200
7	300

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

12.3 Incubate the microtiter plate at room temperature for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.

12.4 Following incubation, aspirate the contents of the wells.

12.5 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.

12.6 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.

12.7 Wash and blot the wells as described in 13.4 - 13.5.

12.8 Pipette 100 µL of Chromogen Substrate Solution into each well.

12.9 Incubate in the dark at room temperature for precisely ten (10) minutes.

12.10 After ten minutes, add 100 µL of Stop Solution to each well.

12.11 Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications.

13. Calculations

13.1 Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.

13.2 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.

13.3 Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the SAA concentration in original samples.

Technical Support

For all technical or commercial enquiries please go to:

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