ab205087 –
Horse SAA ELISA Kit

Instructions for Use

For the quantitative measurement of SAA in horse plasma and serum samples.

This product is for research use only and is not intended for diagnostic use.

Version 7a Last Updated 30 January 2024

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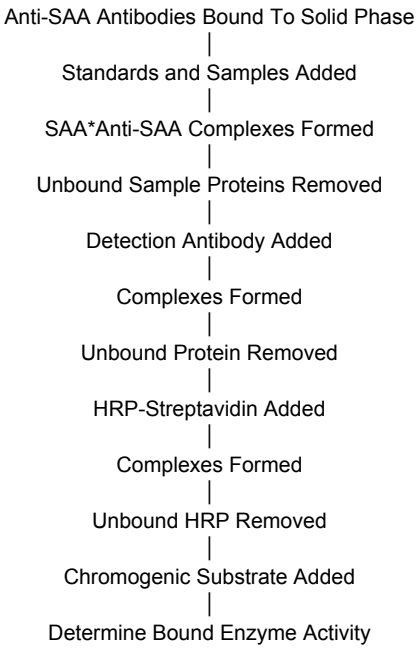
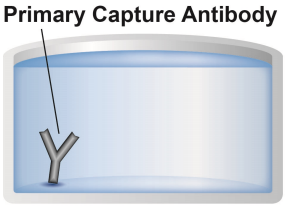
1. BACKGROUND

The Horse SAA (ab205087) test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring SAA in biological fluid of Horses.

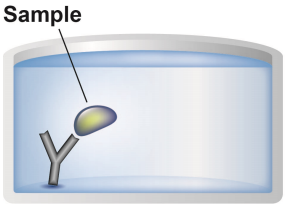
In this assay the SAA present in samples reacts with the anti-SAA antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, the Detection Antibody, biotin conjugated anti-SAA, is added and complexes are formed. Following a wash 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 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.


Anti-SAA Antibodies Bound To Solid Phase
Standards and Samples Added
SAA*Anti-SAA Complexes Formed
Unbound Sample Proteins Removed
Detection Antibody Added
Complexes Formed
Unbound Protein Removed
HRP-Streptavidin Added
Complexes Formed
Unbound HRP Removed
Chromogenic Substrate Added
Determine Bound Enzyme Activity

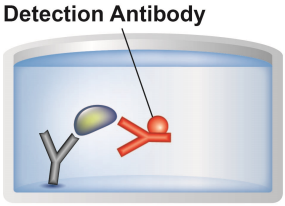
2. ASSAY SUMMARY



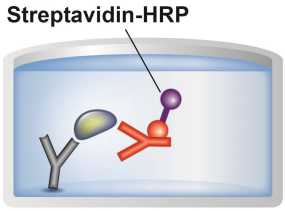
Remove appropriate number of antibody coated well strips. Equilibrate all reagents to room temperature. Prepare all the reagents, samples, and standards as instructed.



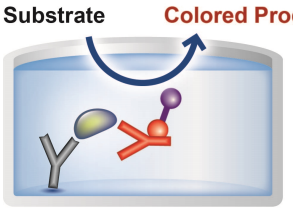
Add standard or sample solution to each well. Incubate at room temperature.



Aspirate and wash each well. Add prepared Biotin labeled detector antibody. Incubate at room temperature.



Aspirate and wash each well. Add prepared HRP-Streptavidin. Incubate at room temperature.



Aspirate and wash each well. Add Chromogen Substrate Solution to each well. Measure immediately.

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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 kit at +4°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 9. Reagent Preparation.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage condition (After Preparation)
5X Diluent Concentrate	50 mL	4°C	4°C
20X Wash Buffer Concentrate	50 mL	4°C	4°C
100X Detection Antibody	150 µL	4°C	4°C*
100X HRP-Streptavidin	150 µL	4°C	4°C*
Chromogen Substrate Solution	12 mL	4°C in the dark	4°C
Stop Solution	12 mL	4°C	4°C
Anti-Horse SAA ELISA Microplate	1 Unit	4°C	4°C
Horse SAA Calibrator (Lyophilized)	1 Vial	4°C	-20°C

*** Only dilute immediately prior to use**. The working conjugate solution is stable for up to 1 hour when stored in the dark.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate washer/ aspirator
- Precision pipettes to deliver 2 µL to 200 µL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- Timer
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.
- Assorted glassware for the preparation of reagents and buffer solutions.
- Orbital Shaker

7. 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.

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.
- Once your standard has been reconstituted, it should be used right away or else 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.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Scientific Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 **5X Diluent Concentrate**

The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH2O.

9.2 **20X Wash Buffer 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 dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. If crystals are observed, warm the buffer concentrate to 30-35°C to dissolve the crystals before dilution.

9.3 **100X Detection Antibody**

Dilute immediately before use and protect from light. Calculate the required amount of 1X Detection Antibody solution for each microplate 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 and avoid foaming.

9.4 **100X HRP-Stretavidin**

Dilute immediately before use and protect from light. Calculate the required amount of 1X HRP-Streptavidin solution for each microplate 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 and avoid foaming.

9.5 **Chromogen Substrate Solution**

Ready to use as supplied.

Stop Solution

Ready to use as supplied.

9.6 **Anti-Horse SAA ELISA Microplate**

Ready to use as supplied. Unseal Microplate pouch and remove plate. Store stripwells that will not be used in this experiment by placing back in pouch and re-seal with the supplied desiccant.

9.7 **Horse SAA Calibrator**

Add 1 mL of distilled or de-ionized water to the Horse SAA Calibrator 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 frozen if future use is intended). Horse SAA Calibrators need to be prepared immediately prior to use. Mix well between each step. Avoid foaming. Store at -20°C long term (stable for up to 14 days at 4°C).

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.

10.1 The horse SSA Calibrator should be aliquoted and stored at -20°C. Avoid multiple freeze-thaw cycles. The calibrator is provided at the weight stated on the vial.

10.2 Label tubes numbers 1-7.

10.3 Prepare a (1/100 dilution) Standard A solution by adding the appropriate volume of 1X Diluent Solution to a tube labelled A, along with the appropriate volume of liquid horse SAA Calibrator. Mix thoroughly but gently.

10.4 Prepare Standard #1 by adding the appropriate volume of 1X Diluent Solution and Standard A (derived below) to tube #1.

*Example:

NOTE: This example is for demonstration purposes only. Please remember to check your calibrator vial for the actual concentration of calibrator provided.

C_S = Starting concentration of reconstituted horse SSA Calibrator (variable e.g. 42.3 µg/mL/42,300 ng/mL)

C_F = Final concentration of horse SSA Calibrator for the assay procedure (e.g. 100 ng/mL)

V_A = Total volume of stock horse SSA Calibrator to dilute (e.g. 2 µL)

V_D = Total volume of 1X Diluent Solution required to dilute horse SSA Calibrator to prepare **Standard #1**

V_T = Total volume of **Standard #1**

Calculate the dilution factor (D_F) between stock calibrator and the **Standard #1** final concentration:

$$Cs/C_F = D_F$$

$$42{,}300 / 100 = 423$$

Calculate the final volume V_D required to prepare the **Standard #1** at 800 ng/mL

$$V_A * D_F = V_T$$

$$V_D = V_T - V_A$$

$$2 * 423 = 846\ \mu L$$

$$V_D = 846 - 2 = 844\ \mu L$$

To tube #1, add 10 µL of horse SAA Calibrator to 595 µL of 1X Diluent Solution to obtain a concentration of 72 ng/mL (**Standard #1**).

10.5 Add 300 µL 1X Diluent Solution into tube numbers 2-7.

10.6 Prepare **Standard #2** by adding 300 µL Standard #1 to tube #2. Mix thoroughly and gently.

10.7 Prepare **Standard #3** by adding 300 µL from **Standard #2** to #3. Mix thoroughly and gently.

10.8 Using the table below as a guide to prepare further serial dilutions.

10.9 1X Diluent Solution serves as the zero standard (0 ng/mL).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Final Conc. (ng/mL)
1	See step 10.3			72
2	300	300	600	36
3	300	300	600	18
4	300	300	600	9
5	300	300	600	4.5
6	300	300	600	2.25
7	0	600	600	0.0

11. SAMPLE COLLECTION AND STORAGE

11.1 **Serum** – Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation.

11.2 **Plasma** – 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 freeze-thaw cycles.

- **Precautions**

  For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

- **Additives and Preservatives**

  No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid contamination with sodium azide.

12. SAMPLE PREPARATION

- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.
- The assay for quantification of SAA in samples requires that each test sample be diluted before use. For a single step determination, dilution of 1/200 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard 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.

13. PLATE PREPARATION

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

14. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

14.1 Pipette 100 µL of each standard, including zero control, in duplicate, into the designated wells.

14.2 Pipette 100 µL of sample (in duplicate) into the designated wells.

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

14.4 Following incubation, aspirate the contents of the wells.

14.5 Completely fill each well with appropriately diluted 1X Wash Buffer 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 gently striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

14.6 Pipette 100 µL of appropriately diluted Detection Antibody to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

14.7 Wash and blot the wells as described in 14.4 - 14.5.

14.8 Pipette 100 µL of appropriately diluted HRP-Streptavidin to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

14.9 Wash and blot the wells as described in 14.4 - 14.5.

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

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

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

14.13 Immediately measure the absorbance of the plate at 450 nm using a microplate reader. Calibrate the plate reader to manufacturer's specifications, if necessary.

Note: *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*

15. CALCULATIONS

15.1 Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

15.2 Create a standard curve by plotting the blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis). Individual absorbance values or average absorbance values can be plotted based on the graphing program used. Draw the best smooth curve through these points to construct the standard curve.

Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is typically the best choice however, other algorithms can be examined to see which can best fit the plotted values (e.g. linear, semi-log, log/log, 4 parameter logistic).

15.3 Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the determined target concentration by the appropriate sample dilution factor as needed.

15.4 Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

16. TYPICAL DATA

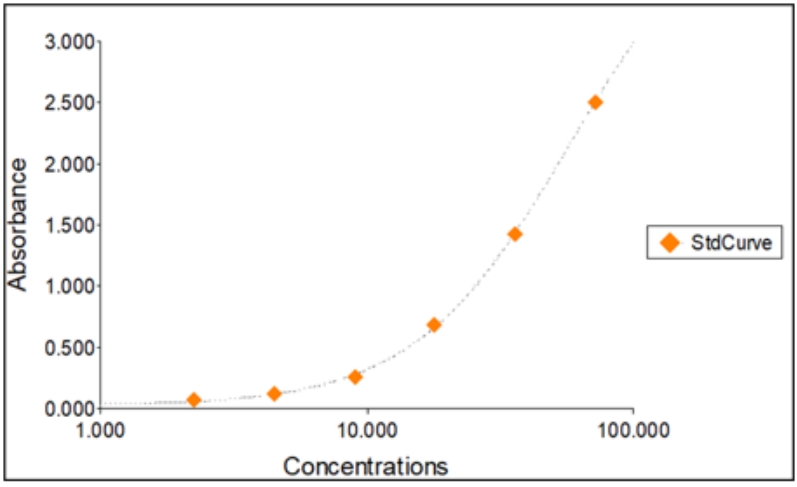

Figure 1: Sample Standard Curve. A standard curve should be generated each time the test is performed.

Concentration (ng/mL)	Background Adjusted OD Value
2.25	0.067
4.5	0.120
9	0.263
18	0.682
36	1.431
72	2.508

17. TYPICAL SAMPLE VALUES

SENSITIVITY -

Calculated detectible range = 2.25 ng/mL – 72 ng/mL

RECOVERY -

Control Serum Recovery = > 85%

PRECISION –

	Intra-Assay	Inter-Assay
%CV	**<10%**	**<10%**

18. INTERFERENCES

These chemicals or biologicals will cause interferences in this assay causing compromised results or complete failure:

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipette performance
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation time; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
High % CV	Inaccurate pipetting	Check pipette performance
	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, ensure it is working properly.

20. NOTES

Technical Support

For all technical or commercial enquiries please go to:
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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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