

# LifeAssays<sup>®</sup> Equine Serum Amyloid A (eSAA) Test Kit

E-25-0024-04

## Intended Use

The LifeAssays<sup>®</sup> Equine serum amyloid A (SAA) Test Kit is an *in vitro* veterinary diagnostic test for the quantitative determination of amyloid A in equine serum. It is intended to be used as an aid in the diagnosis, monitoring and therapy of various inflammatory conditions in horses.

## Introduction

An increased concentration of serum amyloid A (SAA), a major equine acute phase protein, is the most sensitive method for detecting systemic inflammation in horses<sup>1</sup>. Healthy horses have a very low serum SAA concentration, whereas there is a very rapid and large increase in it following an inflammatory stimulus<sup>2,3,4</sup>. Serum SAA levels increase rapidly after the onset of an inflammatory stimulus (6-12 hours)<sup>12</sup> and then, due to SAA's short serum half-life<sup>5</sup>, serum concentrations fall rapidly during recovery. SAA is a non-specific inflammatory marker and increased serum concentrations are seen in bacterial infections<sup>3,6,7,8</sup>, viral infections<sup>2,9</sup> and sterile inflammatory conditions<sup>10</sup>. Increases in serum SAA concentrations have been shown to correlate with the severity of clinical signs<sup>8</sup> and surgical trauma<sup>11</sup>.

## Content Information

Foil pouches each containing a disposable

LifeAssays <sup>®</sup> eSAA Cartridge (Art.No. 40101-26)	
and a disposable pipette (Art.No. 40301-37)	20 pcs
Sample dilution buffer vials (Art.No. 40301-36)	20 pcs
Disposable end-to-end 5 µL glass capillaries (Art.No. 40301-03)	25 pcs
Lot specific calibration key (Art.No. 50501-11)	1 pcs
Instructions for Use (Art.No. 40301-38)	1 pcs

## Material Required but Not Supplied with Kit

- LifeAssays<sup>®</sup> Magnia Reader (Art.No. 50101-10)
- Capillary holder (Art.No. 40301-04)
- A Vortex mixer, e.g. LifeAssays<sup>®</sup> vortex (Art.No. 50201-04) is not essential, but is recommended

## Stability and Storage

Store the test kit and all components at 2–27° C. The test kit is stable until the expiry date printed on the box. Avoid direct sunlight or exposure to temperatures above 27°C. Do not freeze.

## Test Principle

The LifeAssays<sup>®</sup> eSAA is an immunochromatographic assay utilising superparamagnetic nanoparticles as a label. Testing is performed by adding 3 drops (appr. 75 µL) of diluted sample into the sample well of the LifeAssays<sup>®</sup> eSAA Cartridge. The sample flows along a nitrocellulose membrane reacting first with anti-SAA labelled with paramagnetic particles and then the SAA-paramagnetic antibody complexes are captured by an anti-SAA antibody in a capture/reading zone. After 5 minutes incubation, the captured paramagnetic particles are read in the Magnia<sup>®</sup> Reader unit. See assay procedure below for more information. The Magnia<sup>®</sup> Reader calculates the SAA concentration of the sample and presents the result on the device's display.

The linear range of the LifeAssays<sup>®</sup> eSAA assay is 10-1000 mg/L.

Each LifeAssays<sup>®</sup> Equine SAA Test Kit is delivered with a lot-specific Calibration Key. The key contains all the reagent data and parameters for a self-executable algorithm, which will calculate and present the eSAA test results in mg/L. The Calibration Key and the LifeAssays<sup>®</sup> eSAA Cartridge must have same lot number!

## Warnings and Precautions

- For *in vitro* veterinary diagnostic use only.
- Do not mix components from different kit containers.
- Do not use test kits past their expiry date.
- Do not use damaged or contaminated kits.
- Bring all test kit components to room temperature (18-25 °C or 64-77 F) before use.
- The Sample Dilution Buffer contains sodium azide in concentrations < 0.1% as a preservative. Sodium azide is a toxic agent. Avoid contact with skin and eyes. Flush abundantly with water upon disposal or if direct contact occurs.
- Wear disposable gloves while handling samples, kit reagents, and wash hands thoroughly afterwards.
- Disposal of all specimen and test material should be in accordance with state and local law.
- If the instructions for use are not carefully followed, erroneous results may be obtained! The LifeAssays<sup>®</sup> eSAA Assay shall be used only for the *in-vitro* quantification of SAA in equine serum or plasma samples according to the instructions for use. General laboratory procedures and precautions must be obeyed in handling and disposal of samples and test materials.
- Do not re-use LifeAssays<sup>®</sup> eSAA Cartridges. Do not use expired Cartridges from a lot not showing proper performance when tested with the controls. Do not use a Cartridge from a pouch which has been damaged during storage.

## Specimen Collection and Preparation

The LifeAssays<sup>®</sup> Equine SAA test can be performed on both serum and EDTA plasma samples. A normal venous blood sample should be taken and the serum or plasma separated appropriately. If not to be tested immediately, serum or plasma can be stored at 2-8 °C for up 35-46 F) to ten (10) days or at -20 °C (-4 F) for one month.

**Note:** *Repeated freezing and thawing of samples should be avoided. All samples must reach room temperature (18-25 °C or 64-77 F) before testing. Frozen samples must be thawed completely, mixed thoroughly and brought to room temperature before testing is carried out.*

## Interferences

Free hemoglobin or lipids in the sample do not interfere with test results.

## Assay Procedure

### Important Procedural Notes!

- All kit components must be allowed to reach room temperature (18-25 °C or 64-77 F) before use.
- Always use a new and unbroken capillary. Avoid handling the capillary directly with your hands and without gloves (use of capillary holder is recommended).
- Sample dilution buffer may stick to the caps of the vials during transport. Shake or tap vials to remove the buffer from the cap prior to use. To avoid loss of buffer, never open a vial that has buffer caught in the cap.
- The procedural steps should be performed successively without any interruptions.
- Sample dilution buffer vials should be closed tightly after use.
- For more information, see the Magnia<sup>®</sup> Reader's operator manual.

## Assay Steps

**1. Turn on the Magnia<sup>®</sup> Reader by pressing “Power”.** After few seconds, the Reader prompts: “Follow test kit instructions” and then: “Insert Test”. The Magnia<sup>®</sup> Reader is now ready. Please note, that if it is not used within 20 minutes the Reader will turn itself off.

**2. Open the aluminium test pouch having one LifeAssays<sup>®</sup> Cartridge and one disposable pipette inside. Insert the LifeAssays<sup>®</sup> Cartridge into the Reader so that the small arrow on the end of the Cartridge is visible and pointing towards you. The sample well marked with “S” will remain outside the Reader.** The Reader prompts: “Use Calibration Key” and then: “Insert Card”.

**3. Insert the lot-specific LifeAssays<sup>®</sup> Equine SAA “calibration key” card into the Reader.** The card is inserted into the slot behind the display so that the text on the key card remains upwards and visible. The Reader then prompts by displaying the Test Name “eSAA”, the LOT number and Expiration date and then prompts: “Press MEASURE”. The Reader is now ready for the assay. Follow the Test Instructions and do not press “MEASURE” until assay step 7 (see below).

**Note:** Ensure that the Test name, the LOT number and the Expiration dates on the display, the Calibration Key and the aluminium test pouch are identical! Ensure also, that the Expiration date has not yet passed.

**4. Fill the provided glass capillary completely with of serum or plasma while holding the capillary horizontally.**

**Note:** Use the capillary holder (sold as an accessory) for capillary handling. Avoid the introduction of air bubbles into the glass capillary. Be sure to fill the entire length of the capillary with serum or plasma. Avoid excess sample on the outside of the capillary by “wiping off” excess fluid with a clean tissue.

**5. Place the capillary into a sample dilution buffer vial and mix by inverting the tube until the serum or plasma has been completely eluted from the capillary and mixed with the buffer.** Note that complete mixing requires inverting the tube at least 30 times. **Tip:** For easier and quicker mixing use the LifeAssays Vortex.

**5a. Optionally, instead of using the capillary, add 5 µL of serum or plasma into the buffer vial with a micropipette.** Please ensure that no additional serum or plasma is transferred into the buffer vial e.g. via the pipette tip.

**6. After mixing, proceed without any delay to step 7.**

**7. Aspirate the diluted sample with the pipette provided and add 3 drops to the sample well (round opening in the Cartridge) marked with “S” and immediately press “MEASURE”.**

**8. The instrument will incubate the LifeAssays<sup>®</sup> Cartridge for 5 minutes.** The Reader prompts: “Processing...”, “Please wait...” and counts down the seconds. After 5 minutes the Reader will read and calculate the result. Note, that if the Reader is unused for over 20 min, it will turn itself off. In that case, the last measured result is not lost but will be displayed when the Reader is powered up again.

**9. The Magnia<sup>®</sup> Reader compares the signal intensity of the reading zone of the Cartridge to the internal calibration standard and reports the final result as mg/L.** The Reader displays the quantitative test result and prompts: “Remove Test”. The assay is now complete and the result can be recorded.

**10. Read the result from the display. Record the result.**

**Note:** The test result will disappear and be permanently lost, when a new LifeAssays<sup>®</sup> Cartridge is inserted into the Reader!

**11. After recording the result, remove the LifeAssays<sup>®</sup> Cartridge from the Reader and check the control line visually.** There should be a pink control line across the window area next to the letter “C” in the plastic housing. If there is no control line, the test result is invalid and should be discarded.

**12. The Reader is now ready for the next measurement.** Start from the stage number 2. Note, that if the Reader is unused for over 20 min, it will turn itself off. In that case, the last measured result will be shown on display after the Reader is powered up.

## Quality Control

The LifeAssays<sup>®</sup> Equine SAA Cartridge has a control line running across the window area next to the letter “C” in the plastic housing. The control line should develop a pink color when the assay is performed. If the control line is not visible after the assay has been performed, discard the result and repeat the assay with a fresh LifeAssays<sup>®</sup> Equine SAA Cartridge.

## Performance Characteristics

**Linear range:** 10-1000 mg/L

**Correlation:**  $r^2 = 0.95$  vs. Eiken Serum Amyloid A test

**Imprecision:** CV = 7%

## Reference Range

We recommend that Equine SAA results over 20 mg/L should be regarded as an indication of systemic inflammation<sup>12,13</sup>.

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