KAMIYA BIOMEDICAL COMPANY

Bovine SAA3 ELISA

For the quantitative determination of SAA3 in bovine milk

Cat. No. KT-1889

For Research Use Only.

PRODUCT INFORMATION
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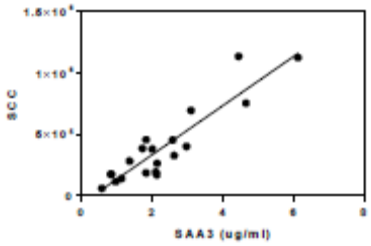
PRODUCT
The **K-ASSAY®** Bovine SAA3 ELISA is an enzyme immunoassay for the quantitative determination of SAA3 in bovine milk. For research use only.

INTRODUCTION
SAA3, is the isoform of serum amyloid A that is expressed in bovine mammary epithelial cells and secreted into milk. It is a positive acute phase protein of ≈12 kDa. Levels increase because of inflammation and infection associated with mastitis. In studies, we find that milk SAA3 levels correlate with somatic cell count (SCC).



PRINCIPLE
The assay uses two bovine SAA3 monoclonal antibodies for solid phase immobilization (microtiter wells) and HRP conjugated for detection. Diluted milk samples and calibrators are incubated in microtiter wells together with HRP conjugate for one hour. If present, SAA3 molecules are sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If SAA3 is present, a blue color develops. Color development is stopped by addition of Stop solution; changing the color to yellow. Absorbance is measured at 450 nm. The concentration of SAA3 is proportional to absorbance and is derived from a calibration curve.

COMPONENTS
- Anti-SAA3 coated plate (12 x 8-well strips)
- HRP conjugate: 11 mL
- SAA3 calibrator stock **Store ≤ -20℃**
- 20X Wash solution: 50 mL
- Diluent: 2 x 50 mL
- TMB: 11 mL
- Stop solution: 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Pipettors and tips
- Distilled or deionized water
- Polypropylene tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE
The SAA3 calibrator stock should be stored at or below -20℃. The remainder of the kit should be stored at 4℃. The microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable until the expiration date stated on the labels.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use, dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water. Unused wash buffer may be stored at 4°C for one week.

DILUENT PREPARATION

The diluent is specially formulated for measurement of SAA3 in bovine milk. It is provided ready to use. Do not substitute other buffers.

CALIBRATOR PREPARATION

1. The calibrator stock consists of 3 µg/mL of pure SAA3 in a stabilizing matrix. Prepare the 30 ng/mL calibrator by mixing 10.0 µL of stock with 0.99 mL of diluent.
2. Label four polypropylene tubes as 15, 7.5, 3.75 and 1.875 ng/mL. Dispense 0.5 mL of diluent into each.
3. Pipette 0.5 mL of the 30 ng/mL SAA3 calibrator into the tube labeled 15 ng/mL and mix. This provides the 15 ng/mL SAA3 calibrator.
4. Similarly prepare the remaining calibrators by two-fold serial dilution.

Use the calibrators within 30 minutes. Although the SAA3 calibrator stock is stable for several days at room temperature, it should be frozen for optimum stability.

SAMPLE PREPARATION

This kit was designed specifically for measurement of SAA3 in milk. In milk with SCC in the range of 0.06 to 1.1 x 10^6 we found SAA3 levels ranging from 0.5 to 6 µg/mL. Levels as high as 40 µg/mL were found in milk from samples with severe mastitis. We suggest testing milk of normal appearance at a dilution of 400-fold, but optimum dilutions should be determined empirically. To avoid matrix effects, do not test milk at dilutions less than 100-fold. A 400-fold dilution can be obtained as follows.

1. Dispense 90 µL and 487.5 µL of diluent into separate microcentrifuge tubes.
2. Mix 10 µL of milk with 90 µL of diluent in the first tube. This represents a 10-fold dilution.
3. Prepare a 400-fold dilution of the sample by mixing 12.5 µL of the 10-fold diluted sample with 487.5 µL of diluent in the second tube.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Unused strips should be stored in a sealed bag with desiccant at 4°C.
2. Dispense 100 µL of calibrators and samples into the wells (we recommend that calibrators and samples be run in duplicate).
3. Add 100 µL of HRP-conjugate to each well.
4. Incubate on a plate shaker at 150 rpm and 25°C for one hour.
5. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µL/well).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Dispense 100 µL of TMB into each well.
8. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
9. After 20-minutes, stop the reaction by adding 100 µL of Stop solution to each well.
10. Gently mix. It is important to make sure that all the blue color changes to yellow.
11. Read absorbance at 450 nm with a plate reader within 5 minutes.

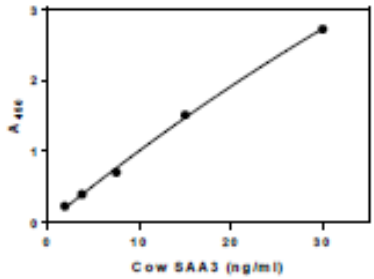
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus SAA3 concentration.
2. Fit the calibration curve using graphing software. We typically fit to a single site binding (hyperbola) model.
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the milk sample.
4. If the A_{450} values of samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with absorbance at 450 nm on the Y-axis against SAA3 concentrations on the X-axis is shown below. This curve is for illustration only.

SAA3 (ng/ml)	A_{450}
30	2.719
15	1.511
7.5	0.702
3.75	0.390
1.875	0.223

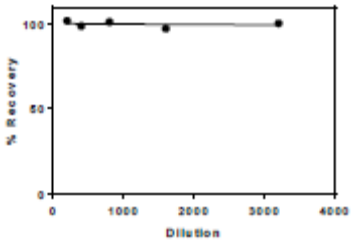


PERFORMANCE

Inter-Assay Precision (Precision between assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Inter-Assay Precision			
Sample	1	2	3
n	3	3	3
Average (µg/ml)	0.111	6.43	30.39
Std. Deviation	0.005	0.54	0.75
CV%	4.7	8.4	2.5

Linearity: To assess the linearity of the assay, a milk sample containing SAA at a concentration of 6.1 µg/mL was serially diluted with diluent to produce values within the dynamic range of the assay.



FOR RESEARCH USE ONLY

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