

Cow IgA ELISA

Life Diagnostics, Inc., Catalog Number: IGA-11

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INTRODUCTION

This ELISA kit is designed for measurement of IgA in cow serum, plasma, or milk. The assay uses sheep anti-cow IgA for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated sheep anti-cow IgA for detection. Both capture and detection antibodies react specifically with cow IgA. Cross-reactivity with immunoglobulins from other species has not been investigated.

PRINCIPLE OF THE ASSAY

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside cow IgA standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgA molecules are thus sandwiched between the immobilization and detection antibodies. After washing the wells to remove unbound HRP-conjugate, TMB reagent is added and incubated for 20 minutes. This results in the development of a blue color. Color development is stopped by the addition of Stop solution, changing the color to yellow. Optical density is measured at 450 nm. The concentration of IgA is proportional to the optical density of the test sample and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti cow IgA coated 96-well plate **Store $\leq -20^{\circ}\text{C}$**
- HRP conjugate stock, 75 μl **Store $\leq -20^{\circ}\text{C}$**
- Cow IgA stock (lyophilized), 3 vials **Store $\leq -20^{\circ}\text{C}$**
- 20x Wash solution: TBS50-20, 50 ml
- Diluent: CSD50-1, 50 ml
- TMB reagent: TMB11-1, 11 ml
- Stop solution (1N HCl): SS11-1, 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-plate incubator/shaker
- Plate washer
- Plate reader with an OD range of 0-4 at 450 nm
- PC graphing software or graph paper

STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored appropriately. Store the 96-well plate, IgA stock and HRP conjugate stock vials at or below -20°C . Store the remaining components in the refrigerator at $2-8^{\circ}\text{C}$. The microtiter plate should always be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents except the HRP stock should be allowed to reach room temperature (25°C) before use.
3. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

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 deionized water.

STANDARD PREPARATION

1. Reconstitute the cow IgA stock as detailed on the vial label. Vortex or mix to ensure complete reconstitution. **The reconstituted standard is stable at $2-8^{\circ}\text{C}$ for one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended.**
2. Label 6 polypropylene or glass tubes as 1000, 500, 250, 125, 62.5 and 31.25, ng/ml.
3. Into the tube labeled 1000 ng/ml, pipette the volume of diluent detailed on the IgA stock vial label. Then add the indicated volume of IgA stock and mix gently. This provides the 1000 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labelled 500, 250, 125, 62.5 and 31.25 ng/ml.
5. Prepare a 500 ng/ml standard by diluting and mixing 250 μl of the 1000 ng/ml standard with 250 μl of diluent in the tube labeled 500 ng/ml.
6. Similarly prepare the remaining standards by serial dilution.

SAMPLE PREPARATION

We found that IgA is present in normal cow serum and milk at concentrations of $\approx 400 \mu\text{g/ml}$. In order to obtain values within range of the standard curve we suggest that samples initially be diluted 1000-fold using the following procedure.

1. Dispense 95 μl and 245 μl of diluent into two tubes.
2. Pipette and mix 5.0 μl of the sample into the first tube containing 95 μl of diluent. This provides a 20-fold dilution.
3. Mix 5.0 μl of the 20-fold diluted sample with the 245 μl of diluent in the second tube. This provides a 1000-fold dilution of the sample.

When testing milk, samples should be diluted at least 1000-fold. At lower dilutions (i.e., 500-fold) a matrix effect that produces false low values occurs.

HRP CONJUGATE PREPARATION

The HRP conjugate should be prepared approximately five minutes before required. The HRP conjugate stock should be diluted with diluent as detailed on the stock vial label.

PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Store unused strips at -20°C in a sealed plastic bag with desiccant.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C¹ for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove residual wash buffer.
6. Add 100 µl of diluted HRP conjugate into each well.
7. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 45 minutes.
8. Wash as detailed in steps 4 to 5 above.
9. Dispense 100 µl of TMB reagent into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

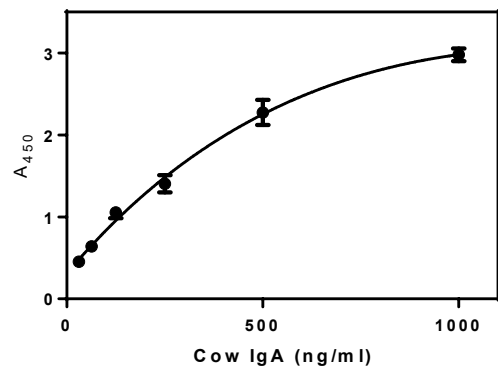
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgA in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgA in the sample.
5. PC graphing software should be used for the above steps if available. We recommend a fit using a second order polynomial equation or a single site, total and nonspecific binding equation.
6. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450 nm on the Y-axis against IgA concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. A standard curve should be generated in each experiment.

IgA (ng/ml)	A_{450}
1000	2.978
500	2.275
250	1.404
125	1.053
62.5	0.640
31.25	0.453



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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For technical assistance please email us at
techsupport@lifediagnosics.com

¹The ELISA was validated using a shaking incubator at 150 rpm and 25°C. Operation of the assay at lower temperatures and mixing speeds will likely give lower absorbance values.