Cow IgG ELISA
Life Diagnostics, Inc., Catalog Number: IGG-11

Cow IgG ELISA

INTRODUCTION
This ELISA kit is designed for measurement of IgG in cow serum, plasma and milk. The assay uses sheep anti-cow IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated sheep anti-cow IgG for detection. Both capture and detection antibodies react specifically with cow IgG. 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 IgG standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgG 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, and optical density is measured at 450 nm. The concentration of IgG 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 IgG coated 96-well plate Store ≤ -20°C
- HRP conjugate stock, 50 μl Store ≤ -20°C
- Cow IgG stock (lyophilized), 3 vials Store ≤ -20°C
- 20x Wash solution, 50 ml
- 10x Diluent (CSD25-10), 25 ml
- TMB reagent, 11 ml
- Stop solution (1N HCl), 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, HRP conjugate stock and IgG stock vials at or below -20°C. Store the remaining components in the refrigerator at 2-8°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.

DILUENT PREPARATION
The dilution buffer is provided as a 10x stock. Determine the volume of diluent required for your assay and dilute 1 volume of 10x diluent with 9 volumes of distilled or deionized water.

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 IgG stock as detailed on the vial label. Vortex or mix to ensure complete reconstitution. The reconstituted standard is stable at 2-8°C for one day.
2. Label 6 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.63 and 7.81 ng/ml.
3. Into the tube labeled 250 ng/ml, pipette the volume of diluent detailed on the IgG stock vial label. Then add the indicated volume of IgG stock and mix gently. This provides the 250 ng/ml standard.
4. Dispense 250 μl of diluent into the tubes labelled 125, 62.5, 31.25, 15.63 and 7.81 ng/ml.
5. Prepare a 125 ng/ml standard by diluting and mixing 250 μl of the 250 ng/ml standard with 250 μl of diluent in the tube labeled 125 ng/ml.
6. Similarly prepare the remaining standards by serial dilution.

SAMPLE PREPARATION
Serum & Plasma. We found that IgG is present in normal cow serum at concentrations of ~30 mg/ml. In order to obtain values within range of the standard curve we suggest that samples initially be diluted 300,000 fold using the following procedure.
1. Dispense 998 μl and 898.5 μl of 1x diluent into two tubes.
2. Pipette and mix 2.0 μl of the sample into the first tube containing 998 μl of diluent. This provides a 500 fold dilution.
3. Mix 1.50 μl of the 500 fold diluted sample with the 898.5 μl of diluent in the second tube. This provides a 300,000 fold dilution of the sample.

Milk. We found that normal milk contained IgG at concentrations of ~0.3 mg/ml. In order to obtain values within range of the standard curve we suggest that samples initially be diluted 4,000 fold using the following procedure.
1. Dispense 198 μl and 437.5 μl of 1x diluent into two tubes.
2. Pipette and mix 2.0 μl of the sample into the first tube containing 198 μl of diluent. This provides a 100 fold dilution.
3. Mix 12.5 μl of the 100 fold diluted sample with the 437.5 μl of diluent in the second tube. This provides a 40,000 fold dilution of the sample.

**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.

**ASSAY 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°C 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 IgG in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgG 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 IgG 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.

<table>
<thead>
<tr>
<th>IgG (ng/ml)</th>
<th>( A_{450} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>3.334</td>
</tr>
<tr>
<td>125</td>
<td>2.398</td>
</tr>
<tr>
<td>32.5</td>
<td>1.275</td>
</tr>
<tr>
<td>31.25</td>
<td>0.655</td>
</tr>
<tr>
<td>15.63</td>
<td>0.425</td>
</tr>
<tr>
<td>7.81</td>
<td>0.319</td>
</tr>
</tbody>
</table>

**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.

Rev 071515

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

---

\(^1\)The 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.