**Quantitative Ovine IgG ELISA Test Kit**

Enzyme Linked Immunosorbent Assay for Determination of IgG in Ovine samples

*Cat. No. OG-ELISA-001*

96 TESTS

For Research, *in vitro* Use Only, NOT for Diagnostic Purposes

*Please Read this Package Insert Completely Before Using this Product*

**INTENDED USE**
The Ovine IgG test kit is a highly sensitive sandwich ELISA for measuring IgG in ovine biological samples.

**PRINCIPLE OF THE ASSAY**
The test kit for the detection of IgG in ovine biological samples is designed in the format of a double antibody sandwich ELISA. In this test system the IgG present in samples will bind onto the wells of the microtiter plate supplied pre-coated with anti-IgG antibodies. The bound IgG is detected by using horseradish peroxidase (HRP) conjugated anti-IgG antibodies, which in turn is visualised by adding 3,3',5,5'-tetramethylenediamine (TMB) solution. Any coloured product is measured at 450 nm after adding stop solution. The quantity of bound HRP varies directly with the concentration of IgG in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of IgG in the test sample. The quantity of IgG in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

**CONTENTS**
1. ELISA MICROTITRE PLATE COATED WITH ANTI-OVINE IgG ANTIBODIES (ready-to-use)
   Twelve removable 8-well microtiter strips in well holder frame.
2. ASSAY DILUENT CONCENTRATE
   Two bottles containing 15ml of a 30× concentrated diluent running buffer.
3. WASH BUFFER CONCENTRATE
   One bottle containing 30ml of a 30× concentrated wash buffer.
4. OVINE IgG STANDARD CONCENTRATE
   One vial containing 200µl of Ovine IgG standard concentrate at concentration of 100µg/ml.
5. HRP-ANTIBODY CONJUGATE (ready-to-use)
   One bottle containing 15ml of anti-Ovine IgG antibody conjugated with HRP in a stabilising buffer.
6. TMB SUBSTRATE SOLUTION (ready-to-use)
   One bottle containing 15ml of TMB and hydrogen peroxide in a buffer.
7. STOP SOLUTION (ready-to-use)
   One bottle containing 15ml 0.3M sulphuric acid.

**WARNING:** Avoid contact with skin.

8. MICROTITRE PLATE SEALER
   One piece used to cover the microtiter plate.

**REAGENT PREPARATION**

1. **ASSAY DILUENT (1×)**
The Assay Diluent Concentrate (30×) must be diluted 1/30 before use by mixing 1 part the concentrate with 29 parts distilled or deionised water (dH₂O).

2. **WASH BUFFER (1×)**
The Wash Buffer Concentrate (30×) must be diluted 1/30 before use by mixing 1 part the concentrate with 29 parts dH₂O.

3. **OVINE IgG STANDARDS**
The Ovine IgG standard concentrate is supplied at a concentration of 100µg/ml. Ovine IgG standards need to be prepared immediately prior to use (see Table below). Mix well and change tips between each step. Avoid foaming.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Concentration of Ovine IgG (µg/ml)</th>
<th>Volume of Ovine IgG Standard</th>
<th>Volume of Assay Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1000</td>
<td>10µl Standard concentrate</td>
<td>990µl</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>500 µl standard 1</td>
<td>500µl</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>500 µl standard 2</td>
<td>500µl</td>
</tr>
<tr>
<td>4</td>
<td>125</td>
<td>500 µl standard 3</td>
<td>500µl</td>
</tr>
<tr>
<td>5</td>
<td>62.5</td>
<td>500 µl standard 4</td>
<td>500µl</td>
</tr>
<tr>
<td>6</td>
<td>31.25</td>
<td>500 µl standard 5</td>
<td>500µl</td>
</tr>
<tr>
<td>7</td>
<td>15.625</td>
<td>500 µl standard 6</td>
<td>500µl</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td></td>
<td>500µl</td>
</tr>
</tbody>
</table>

**STORAGE AND STABILITY**
The expiration date for the package is stated on the box label.

1. **ELISA MICROTITRE PLATE COATED WITH ANTI-OVINE IgG ANTIBODIES**
   Stable until the expiration date, and should be stored at 2-8°C in sealed foil pouch with desiccant pack.

2. **ASSAY DILUENT**
   The 30× Diluent Concentrate is stable until the expiration date.
The 1× working solution is stable for at least one week from the date of reparation. Both solutions should be stored at 2-8°C. 3. WASH BUFFER
The 30× Wash Buffer Concentrate is stable until the expiration date. The 1× working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 2-8°C. 4. OVINE IgG STANDARD
The standard concentrate supplied should be stored at 2-8°C and is stable until expiration date. The working standards should be prepared just before use and are stable for up to 8 hours. 5. HRP-ANTIBODY CONJUGATE
The ready-to-use conjugate should be stored at 2-8°C and is stable until expiration date. 6. TMB SUBSTRATE SOLUTION
The Substrate Solution should be stored at 2-8°C and is stable until the expiration date.

SPECIMEN COLLECTION AND HANDLING
Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize haemolysis, excessive haemolysis can impact your results. Colostrum sample should be obtained from a quarter of the udder immediately post partum and prior to suckling. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

MATERIALS PROVIDED
See "CONTENTS"

MATERIALS REQUIRED
BUT NOT PROVIDED WITH KIT
• Precision pipette (2µl to 1000µl) and tips for making and dispensing dilutions
• Test tubes
• Microtitre plate washer/aspirator
• Distilled or deionised water
• ELISA microtiter plate reader
• Assorted glassware for the preparation of reagents and buffer solutions
• Timer
• Vortex mixer
• Microtitre plate shaking equipment
• Absorbent pads (tissue)

DILUTION OF SAMPLES
• Ovine colostrum normally contains 40 – 90 mg/ml of IgG. A 1/1,000,000 dilution of ovine colostrum is recommended for initial testing.
• Ovine milk normally contains ~0.5 mg/ml of IgG. A 1/2,000 dilution of ovine milk is recommended for initial testing.
• Ovine serum normally contains 10 – 25 mg/ml of IgG. A 1/400,000 dilution of ovine serum is recommended for initial testing.

To prepare a 1/400,000 dilution of serum sample, transfer 10µl of sample to 3990µl of 1× Assay Diluent. This gives you a 1/400 dilution. Next, dilute the 1/400 sample by transferring 4µl to 3996µl of 1× Assay Diluent. You now have a 1/400,000 dilution of your sample. Mix thoroughly at each stage. It is strongly suggested that a separate tip should be used at each stage in order to avoid carrying over from the 1/400 diluted sample.

TEST PROCEDURE
Allow all reagents to reach room temperature (RT) before use. Label test tubes to be used for the preparation of standards and samples. If the entire 96-well plate will not be used, remove surplus strips and wells from the plate frame and place into the re-sealable foil pouch with desiccant. Seal bag and store at 2-8°C.
TYPICAL STANDARD CURVE
Below is an example of a typical standard curve. Variations will occur from laboratory to laboratory due to pipetting, incubator temperatures, plate readers, etc.

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>OD_{450nm}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>2.014</td>
</tr>
<tr>
<td>500</td>
<td>1.916</td>
</tr>
<tr>
<td>250</td>
<td>1.667</td>
</tr>
<tr>
<td>125</td>
<td>1.212</td>
</tr>
<tr>
<td>62.5</td>
<td>0.730</td>
</tr>
<tr>
<td>31.25</td>
<td>0.415</td>
</tr>
<tr>
<td>15.625</td>
<td>0.216</td>
</tr>
<tr>
<td>0</td>
<td>0.008</td>
</tr>
</tbody>
</table>

LIMITATION OF THE PROCEDURE
1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionised water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature.
3. Do not mix or substitute reagents with those from other lots or sources.

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