Monkey IgG ELISA
Life Diagnostics, Inc., Catalog Number: IGG-3-INT

**INTRODUCTION**

The monkey IgG ELISA kit is designed for the measurement of IgG in monkey serum or plasma. The assay uses two mouse monoclonal antibodies, developed at Life Diagnostics Inc. (LDI), that recognize monkey and human IgG, with no reactivity toward monkey IgM or IgA. Studies at LDI have shown that the kit works for IgG from Rhesus, Cynomolgus, Squirrel Monkey and Baboon. It does not recognize African Green monkey IgG.

**PRINCIPLE OF THE TEST**

Test samples and standards (100 µl) are incubated in the antibody-coated microtiter wells together anti-IgG-HRP conjugate (100 µl) for 90 minutes. The microtiter wells are subsequently washed to remove unbound HRP conjugate and TMB Reagent is added and incubated for 30 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 spectrophotometrically 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 monkey IgG coated 96-well plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 ml
- Reference standard (lyophilized)1
- 20x Wash Solution, 50 ml
- Diluent, 50 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 mixing speed of ~150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450 nm
- Graph paper (PC graphing software is optional)

**STORAGE**

The test kit will remain stable for six months from the date of purchase provided that the components are stored as described above. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

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1 The standard is prepared from human IgG calibrated against pure Rhesus monkey IgG. The kit recognizes human and monkey IgG identically. The use of human IgG as standard allows export of the kit without requirement for CITES documentation.
3. Dispense 100 µl of standards and diluted samples into the wells (we recommend that standards and samples be tested in duplicate).

4. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 90 minutes².

5. 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.

6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.

7. Dispense 100 µl of TMB Reagent into each well.

8. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 30 minutes.

9. 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 the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS
1. Calculate the average absorbance values (A₄₅₀) 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 concentrations by the dilution factor to determine the actual concentration of IgG in the sample.

5. PC graphing software may be used for the above steps. We recommend use of a second order polynomial or two-site binding model for curve fitting.

6. If the OD₄₅₀ 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 450nm 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. Each user should obtain his or her data and standard curve in each experiment.

<table>
<thead>
<tr>
<th>IgG (ng/ml)</th>
<th>A₄₅₀</th>
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<tbody>
<tr>
<td>5000</td>
<td>3.311</td>
</tr>
<tr>
<td>2500</td>
<td>2.545</td>
</tr>
<tr>
<td>1250</td>
<td>1.717</td>
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<tr>
<td>625</td>
<td>0.968</td>
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<td>312.5</td>
<td>0.533</td>
</tr>
<tr>
<td>156.3</td>
<td>0.282</td>
</tr>
<tr>
<td>78.1</td>
<td>0.156</td>
</tr>
</tbody>
</table>

² The kit was validated using a shaking incubator set at 25°C and 150 rpm. If the assay is performed at lower temperatures and/or shaking speeds, lower absorbance values can be expected.