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

Monkey IgG ELISA
(for export)

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. 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: TBS50-20, 50 ml
- Diluent: YD50-1, 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 mixing speed of 150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- 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 refrigerated at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

[1] The standard s 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.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach 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. The IgG standard is provided as a lyophilized stock. Reconstitute with 0.20 ml of distilled or deionized water ***(the reconstituted standard is stable at 4°C for one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended)***.
2. Label 7 polypropylene or glass tubes as 5000, 2500, 1250, 625, 312.5, 156.3 and 78.1 ng/ml.
3. Into the tube labeled 5000 ng/ml, pipette the volume of diluent detailed on the IgG standard vial label. Then add the indicated volume of IgG standard and mix gently. This provides the 5000 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 2500, 1250, 625, 312.5, 156.3 and 78.1 ng/ml.
5. Prepare a 2500 ng/ml standard by diluting and mixing 250 µl of the 5000 ng/ml standard with 250 µl of diluent in the tube labeled 2500 ng/ml.
6. Similarly prepare the 1250, 625, 312.5, 156.3 and 78.1 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: IgG is typically present in monkey serum or plasma at concentrations of ≈15 mg/ml. In order to obtain values within range of the standard curve, we suggest that samples initially be diluted 5000-fold using the following procedure for each sample to be tested:

1. Dispense 198 µl and 245 µl of diluent into separate tubes.
2. Pipette and mix 2 µl of the serum/plasma sample into the tube containing 198 µl of diluent. This provides a 100-fold diluted sample.
3. Mix 5 µl of the 100-fold diluted sample with the 245 µl of diluent in the second tube. This provides a 5,000-fold dilution of the sample.
4. Repeat this procedure for each sample to be tested

Tissue extracts and body fluids other than serum or plasma will likely have lower IgG levels than those found in serum. Optimal dilutions of such samples should be determined empirically. Serum or plasma dilutions less than 4000-fold should not be used because of matrix effects that lead to underestimation of IgG concentrations.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of HRP-conjugate into each well.
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 minutes[2].
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*.

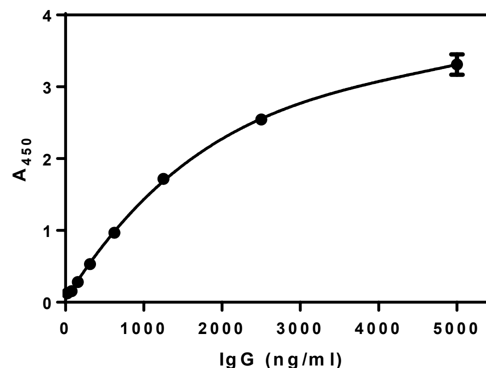
CALCULATION OF RESULTS

1. Calculate the average absorbance values (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 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 OD_{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 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.

IgG (ng/ml)	A_{450}
5000	3.311
2500	2.545
1250	1.717
625	0.968
312.5	0.533
156.3	0.282
78.1	0.156



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@lifediagnostics.com

[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.