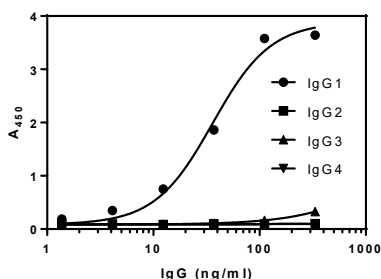


Monkey IgG1 ELISA

Life Diagnostics, Inc., Catalog Number: IGG-3-1

INTRODUCTION

This ELISA kit is designed for measurement of IgG1 in old world monkey serum or plasma. The assay uses monoclonal anti-monkey IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse monoclonal anti-monkey IgG1 antibody for detection. Studies at Life Diagnostics, Inc. have demonstrated that the kit recognizes old world monkey IgG1 and shows no significant reactivity with rhesus monkey IgG2, IgG3 or IgG4 (figure below). The ELISA does not recognize human IgG. Cross-reactivity with immunoglobulins from other species has not been investigated.



PRINCIPLE OF THE TEST

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside monkey IgG1 standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. IgG1 molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP conjugate. TMB Reagent is added and incubated for 20 minutes at room temperature. 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 IgG1 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
- Monkey IgG1 stock (lyophilized)¹
- 20x Wash Solution: TBS50-20, 50 ml
- 10x Immunoglobulin Diluent: RD25-10, 25 ml
- TMB Reagent (One-Step): TMB11-1, 11 ml
- Stop Solution (1N HCl): SS11-1, 11 ml

¹ The reference stock consists of monkey serum in a BSA matrix. The IgG1 content was calibrated using purified recombinant monkey IgG1 as reference material.

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 150rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

STORAGE OF THE TEST KIT

When the kit is received, place the lyophilized IgG1 stock vial in a -20°C to -80°C freezer. **Store all remaining kit components in a refrigerator at 4°C.** The test kit will remain stable for six months from the date of purchase provided that the components are stored as described. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

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

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use, estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (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. The IgG1 stock is provided in lyophilized form. Reconstitute as described on the vial label (**the reconstituted stock is stable at 4°C for one week but should be aliquoted and frozen at -20°C after reconstitution if future use is intended**).
2. Label 5 polypropylene or glass tubes as 100, 50, 25, 12.5, and 6.25 ng/ml.
3. Into the tube labeled 100 ng/ml, pipette the volume of diluent detailed on the IgG1 stock vial label. Then add the indicated volume of IgG1 stock (shown on the vial label) and mix gently. This provides the 100 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 50, 25, 12.5, and 6.25 ng/ml.
5. Prepare a 50 ng/ml standard by diluting and mixing 250 µl of the 100 ng/ml standard with 250 µl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the 25, 12.5, and 6.25 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: IgG1 is present in monkey serum or plasma at concentrations of approximately 10 mg/ml. To obtain values within range of the standard curve, we suggest that samples initially be diluted 250,000-fold using the following procedure for each sample to be tested:

1. Dispense 999 μ l and 498 μ l of 1x diluent into separate tubes.
2. Pipette and mix 1.0 μ l of the serum/plasma sample into the tube containing 999 μ l of diluent. This provides a 1000-fold diluted sample.
3. Mix 2.0 μ l of the 1000-fold diluted sample with 498 μ l of diluent in the second tube. This provides a 250,000-fold dilution of the sample.

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.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μ l of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 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 μ l/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 all residual wash buffer.
6. Add 100 μ l of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at 25°C for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 μ l of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at 25°C for 20 minutes.
11. Stop the reaction by adding 100 μ l 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

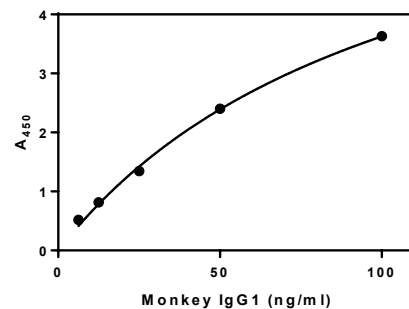
1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgG1 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 may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

7. Do not extend or extrapolate the standard curve beyond the 6.25 and 100 ng/ml standards. Studies at Life Diagnostics, Inc. have demonstrated that the ELISA provides reliable and reproducible results only within this range.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y-axis against IgG1 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.

IgG1 (ng/ml)	A_{450}
100	3.631
50	2.403
25	1.344
12.5	0.816
6.25	0.518



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.

REFERENCES

1. ED Williamson, et al. Immunogenicity of recombinant protective antigen and efficacy against aerosol challenge with anthrax. *Infection and Immunity* 73:5978-5987 (2005)
2. P Procell-Wilkins, et al. Isotypic analysis of humoral immune responses in rhesus monkeys to an adult microsomal antigen of *Schistosoma Mansoni*: an indicator of successful treatment. *Am. J. Trop. Med. Hyg.* 45:629-635 (1991)

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For technical assistance please email us at
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