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

INTRODUCTION

The monkey IgG ELISA kit is designed for measurement of IgG in old world monkey serum or plasma. The assay uses goat anti-monkey IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-monkey IgG antibodies for detection. Both capture and detection antibodies were cross-absorbed on monkey IgM and IgA agarose columns, thereby ensuring specificity for 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 prepared monkey 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. The wells are then washed to remove unbound HRP-labeled antibodies and 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, 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:

- Monkey IgG antibody coated plate (12 x 8 well strips)
- HRP conjugate, 11 ml
- IgG stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- 10x Diluent; YD50-10, 50 ml
- TMB; TMB11-1, 11 ml
- Stop solution; SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 2-8°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

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. Reconstitute the lyophilized stock as detailed on the vial label. Mix gently until dissolved. 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.
1. Label 8 microcentrifuge tubes as 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 0 ng/ml.
2. In 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.
3. Dispense 250 µl of diluent into the tubes labeled 125, 62.5, 31.25, 15.63, 7.81, 3.91 and 0 ng/ml.
4. Prepare the 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.
5. Similarly prepare the 62.5, 31.25, 15.63, 7.81 and 3.91 ng/ml standards by serial dilution.

SAMPLE PREPARATION

IgG is typically present in monkey serum and plasma at concentrations of ≈15 mg/ml. To obtain values within range of the standard curve, we suggest that samples be diluted 100,000-fold using the following procedure for each sample to be tested:

1. Dispense 998 µl and 794 µl of 1x diluent into separate tubes.
2. Pipette and mix 2.0 µl of the serum/plasma sample into the tube containing 998 µl of diluent. This provides a 500-fold diluted sample.
3. Mix 4.0 µl of the 500-fold diluted sample with the 794 µl of diluent in the second tube. This provides a 100,000-fold dilution.

Tissue extracts and body fluids other than serum or plasma will likely have lower IgG levels. Optimal dilutions must be determined empirically.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (standards and samples should be tested in duplicate).
3. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.

4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

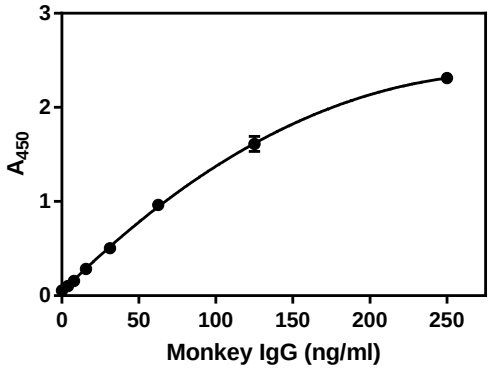
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to an appropriate model and derive the concentration of IgG in the diluted samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to determine the concentration in the original sample.
4. If the A_{450} values of samples fall outside the standard curve, the original samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns. A standard curve must be generated for each experiment.

IgG (ng/ml)	A_{450}
250	2.311
125	1.547
62.5	0.963
31.25	0.503
15.63	0.283
7.81	0156
3.91	0.102
0	0.055



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