KAMIYA BIOMEDICAL COMPANY

Monkey IgG ELISA

For the quantitative determination of IgG in monkey serum or plasma.

Cat. No. KT-468

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Monkey IgG ELISA is an enzyme immunoassay for the quantitative determination of IgG in monkey serum or plasma. For research use only.

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
Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside prepared monkey IgG calibrators. 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 calibration curve.

COMPONENTS
- Anti-monkey IgG -coated microtiter wells, 96 wells
- Monkey IgG Calibrator (lyophilized)
- Diluent (10X), 50 mL
- HRP Conjugate, 11 mL
- Wash Solution (20X), 50 mL
- TMB Reagent (One-step), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED
- Precision pipettes and tips
- Distilled or de-ionized water
- Vortex mixer
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional)
- Polypropylene or glass tubes
- Plate reader with an optical density of 0-4 at 450 nm
- Micro-plate incubator/shaker mixing speed of ~150 rpm
- Plate washer

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 de-ionized 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 de-ionized water.

CALIBRATOR PREPARATION

1. The IgG calibrator is provided as a lyophilized stock. Reconstitute with 1.0 mL of distilled or de-ionized water.
2. Label 8 polypropylene or glass tubes as 1,000, 500, 250, 125, 62.5, 31.25, 15.63 and 0 ng/mL.
3. Into the tube labeled 1,000 ng/mL, pipette 454.66 µL of diluent. Then add 45.34 µL of IgG calibrator and mix gently. This provides the 1,000 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 500, 250, 125, 62.5, 31.25, 15.63 and 0 ng/mL.
5. Prepare a 500 ng/mL calibrator by diluting and mixing 250 µL of the 1,000 ng/mL calibrator with 250 µL of diluent in the tube labeled 500 ng/mL.
6. Similarly prepare the 250, 125, 62.5, 31.25, 15.63 ng/mL calibrators by serial dilution.

Note: The reconstituted calibrator is stable at 4°C for one week but should be aliquoted and frozen at -20°C after reconstitution if future use is intended).

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 calibration curve, we suggest that samples initially 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 µL of the serum/plasma sample into the tube containing 998 µL of 1x diluent. This provides a 500 fold diluted sample.
3. Mix 4 µL of the 500 fold diluted sample with the 794 µL of diluent in the second tube. This provides a 100,000 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.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators 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 room temperature (18-25°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 µL/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 µL of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µL of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
11. Stop the reaction by adding 100 µL 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*.

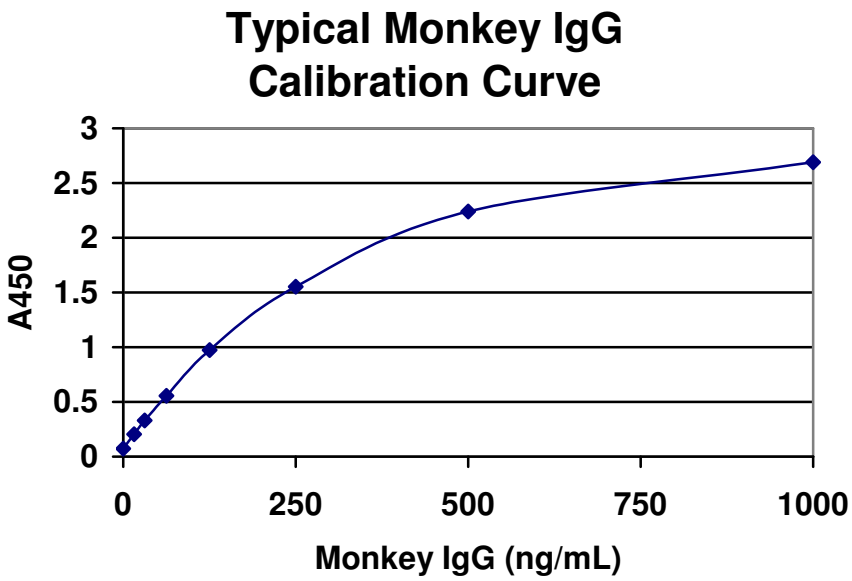
CALCULATION OF RESULTS

1. Calculate the mean absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentration 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 calibration 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.
6. If the OD_{450} values of the sample fall outside the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

Results of a typical calibration run with optical density reading at 450 nm shown on the Y axis against IgG concentration shown on the X axis are illustrated below. This calibration curve is for the illustration purpose only and should not be used to calculate unknowns. A calibration curve should be run for each assay.

IgG (ng/mL)	Absorbance (450 nm)
1,000	2.690
500	2.239
250	1.554
125	0.974
62.5	0.557
31.25	0.330
15.63	0.205
0	0.073



STORAGE

Store kit at 4℃. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air. The expiration date of the kit is indicated on the box label.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (18-25℃) before use.
3. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

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 package insert instructions.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com