KAMIYA BIOMEDICAL COMPANY

Monkey IgG1 ELISA

For the quantitative determination of IgG1 in monkey serum or plasma.

Cat. No. KT-555

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The ***K-ASSAY®*** Monkey IgG1 ELISA is an enzyme immunoassay for the quantitative determination of IgG1 in monkey serum or plasma. For research use only.

INTRODUCTION
This ELISA kit is designed for measurement of IgG1 in old world monkey serum or plasma. The assay uses specific polyclonal anti-monkey IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse monoclonal anti-monkey IgG1 antibody for detection. Studies at KAMIYA BIOMEDICAL COMPANY have demonstrated that the kit recognizes old world monkey IgG1 and shows no reactivity with rhesus monkey IgG2, IgG3 or IgG4. The ELISA does not recognize human 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 monkey IgG1 calibrators. 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 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 IgG1 is proportional to the optical density of the test sample and is derived from a calibration curve.

COMPONENTS
- Anti monkey IgG coated 96-well plate (12 strips of 8 wells)
- Monkey IgG1 stock (lyophilized)
- 10X Immunoglobulin diluent, 25 mL
- HRP Conjugate Reagent, 11 mL
- 20X Wash Solution, 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 range 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 IgG1 stock is provided in lyophilized form. Reconstitute with 1.0 mL of 1X diluent ***(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 calibrator.
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 calibrator by diluting and mixing 250 µL of the 100 ng/mL calibrator with 250 µL of diluent in the tube labeled 50 ng/mL.
6. Similarly prepare the 25, 12.5, and 6.25 ng/mL calibrators by serial dilution.

SAMPLE PREPARATION

General Note: IgG1 is present in monkey serum or plasma at concentrations of ~10 mg/mL, In order to obtain values within range of the calibration curve, we suggest that samples initially be diluted 250,000 fold using the following procedure for each sample to be tested:

1. Dispense 999 µL and 498 µL of 1X diluent into separate tubes.
2. Pipette and mix 1.0 µL of the serum/plasma sample into the tube containing 999 µL of diluent. This provides a 1,000 fold diluted sample.
3. Mix 2.0 µL of the 1,000 fold diluted sample with 498 µL 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 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

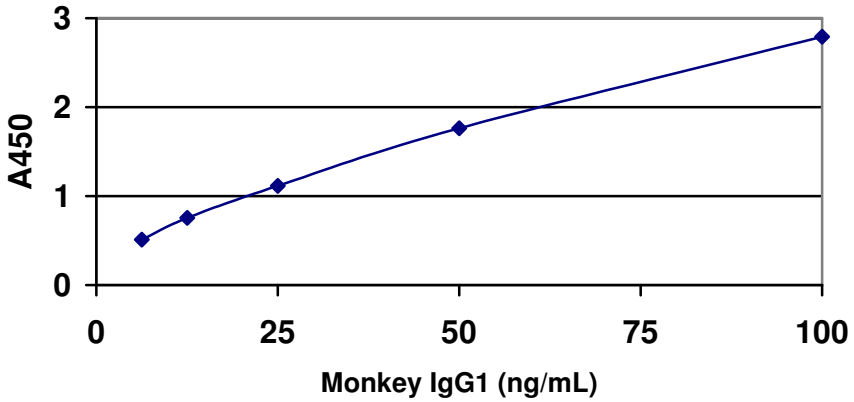
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and 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 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 samples fall outside the calibration curve, samples should be diluted appropriately and re-tested.
7. Do not extend or extrapolate the calibration curve beyond 6.25 and 100 ng/mL. Studies at KAMIYA BIOMEDICAL COMPANY have demonstrated the ELISA provides reliable and reproducible results only within this range.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density readings at 450 nm 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. Each user

should obtain his or her data and calibration curve in each experiment.

IgG1 (ng/mL)	Absorbance (450 nm)
100	2.791
50	1.763
25	1.114
12.5	0.756
6.25	0.510



STORAGE

When the kit is received place the lyophilized IgG1 stock vial in a -20℃ to -80℃ freezer. **Store all remaining kit components in a refrigerator at 4℃.** The test kit will remain stable until the expiration date 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 (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 instructions detailed above.
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