KAMIYA BIOMEDICAL COMPANY

Camel IgG ELISA

For the quantitative determination of IgG in camel serum and plasma

Camel. No. KT-777

For Research Use Only.

PRODUCT INFORMATION

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

INTRODUCTION
Three major IgG isotypes are found in camel: IgG_1, IgG_2 and IgG_3. IgG_1 resembles IgG from other species in that it is comprised of two heavy chains and two light chains. IgG_2 and IgG_3 each consist of two heavy chains but both lack light chains. The heavy chains of IgG_2 and IgG_3 lack the CH1 domain present in IgG_1. The hinge region between the CH2 and VH domain is longer in IgG_2, giving it a slightly higher molecular weight.

This ELISA kit uses anti-camel IgG_3 for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-camel IgG_3 for detection. It recognizes IgG_1, IgG_2 and IgG_3 and can be used for measurement of total IgG in camel serum. Reactivity with IgM is negligible or nonexistent. 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 camel 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-Camel IgG Coated 96-well Plate (12 strips of 8 wells)
- HRP Conjugate Reagent, 11 mL
- Reference Calibrator (lyophilized)
- Wash Buffer (20X), 50 mL
- Diluent (10X), 25 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
- 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 450 nm
- Graph paper (PC graphing software is optional)

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (25°C) before use.

Please read and understand this kit insert before starting your assay. Don't hesitate to contact us by telephone or e-mail should you require technical assistance or clarification.
Optimal 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 de-ionized water.

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.

SAMPLE PREPARATION

General Note: IgG is typically present in camel serum or plasma at concentrations of ~6 mg/mL. In order to obtain values within range of the calibration curve, we suggest that samples initially be diluted 200,000 fold using the following procedure for each sample to be tested:

1. Dispense 998 µL and 798 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 diluent. This provides a 500 fold diluted sample.
3. Mix 2 µL of the 500 fold diluted sample with the 798 µL of diluent in the second tube. This provides a 200,000 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. Optimal dilutions of such samples should be determined empirically.

CALIBRATOR PREPARATION

1. The camel IgG calibrator is provided as a lyophilized stock. Reconstitute with distilled or deionized water as directed on the vial label ***(the reconstituted calibrator 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 8 polypropylene or glass tubes as 75, 37.5, 18.75, 9.38, 4.69, 2.34, 1.17 and 0 ng/mL.
3. Into the tube labeled 75 ng/mL, pipette the volume of diluent detailed on the IgG calibrator vial label. Then add the indicated volume of IgG calibrator and mix gently. This provides the 75 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 37.5, 18.75, 9.38, 4.69, 2.34, 1.17 and 0 ng/mL.
5. Prepare a 37.5 ng/mL calibrator by diluting and mixing 250 µL of the 75 ng/mL calibrator with 250 µL of diluent in the tube labeled 37.5 ng/mL.
6. Similarly prepare the 37.5, 18.75, 9.38, 4.69, 2.34 and 1.17 ng/mL calibrators by serial dilution.

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 HRP 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 concentration by the dilution factor to determine the actual concentration of IgG in the sample.
5. If available, PC graphing software should be used for the above steps. We find that a good fit of the data is obtained with a second order polynomial equation.
6. If the A_{450} values of samples fall outside the range of the calibration curve samples should be re-diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A representative calibration curve with optical density readings at 450 nm on the Y-axis against IgG concentration 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.

IgG (ng/mL)	Absorbance (450 nm)
75	2.515
37.5	1.494
18.75	0.873
9.38	0.507
4.69	0.341
2.34	0.238
1.17	0.199
0	0.155

STORAGE

The kit will remain stable until the expiration date provided that the components are stored at 4℃. It is important that the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

FOR RESEARCH USE ONLY

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