



User's Manual

Camel IgG ELISA Kit



DEIASL056



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

 Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

 Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  Fax: 1-631-938-8221

 Email: info@creative-diagnostics.com  Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

The test kit can be used for measurement of total IgG in camel serum.

General Description

Three major IgG isotypes are found in camel: IgG1, IgG2, and IgG3. IgG1 resembles IgG from other species in that it is comprised of two heavy chains and two light chains. IgG2 and IgG3 each consist of two heavy chains but both lack light chains. The heavy chains of IgG2 and IgG3 lack the CH1 domain present in IgG1. The hinge region between the CH2 and VH domain is longer in IgG2, giving it a slightly higher molecular weight.

Principles of Testing

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside camel 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.

Reagents And Materials Provided

1. Anti Camel IgG Coated 96-well Plate (12 strips of 8 wells)
2. HRP Conjugate Reagent, 11 mL
3. Reference Standard (lyophilized)
4. 20x Wash Solution, 50 mL
5. 10x Immunoglobulin Diluent, 25 mL
6. TMB Reagent (One-Step), 11 mL
7. Stop Solution (1N HCl), 11 mL

Materials Required But Not Supplied

1. Precision pipettes and tips
2. Distilled or deionized water
3. Polypropylene or glass tubes
4. Vortex mixer
5. Absorbent paper or paper towels

6. Micro-plate incubator/shaker mixing speed of ~150 rpm
7. Plate washer
8. Plate reader with an optical density range of 0-4 at 450 nm
9. Graph paper (PC graphing software is optional)

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.

Specimen Collection And Preparation

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

1. Dispense 998 mL and 798 mL of 1x diluent into separate tubes.
2. Pipette and mix 2.0 mL of the serum/plasma sample into the tube containing 998 mL of diluent. This provides a 500 fold diluted sample.
3. Mix 2.0 mL of the 500 fold diluted sample with the 798 mL 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.

Reconstitution And Storage

- 1x Immunoglobulin Diluent: 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.
- 1x Wash Solution: 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.

Reagent Preparation

Standard Preparation

1. The camel IgG standard is provided as a lyophilized stock. Reconstitute with distilled or deionized water as directed on the vial label (the reconstituted standard is stable at 4°C for at least 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 standard vial label. Then add the indicated

volume of IgG standard (shown on the standard vial label) and mix gently. This provides the 75 ng/mL standard.

4. Dispense 250 μ L 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 standard by diluting and mixing 250 μ L of the 75 ng/mL standard with 250 μ L of diluent in the tube labeled 37.5 ng/mL.
6. Similarly prepare 37.5, 18.75, 9.38, 4.69, 2.34 and 1.17 ng/mL standards by serial dilution.

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 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 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 room temperature (18-25°C) for 45 minutes.
8. Wash as detailed in steps 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 room temperature (18-25°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

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 IgG 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. We recommend a fit using a second order polynomial equation.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

Typical Standard Curve



Tel: 1-631-624-4882 (USA)

Tel: 44-161-818-6441 (Europe)



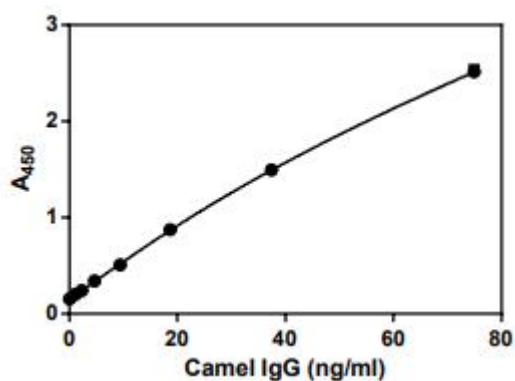
Fax: 1-631-938-8221



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A typical standard curve with optical density readings at 450 nm on the Y-axis against IgG 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. A standard curve should be generated in each experiment.

IgG (ng/ml)	A ₄₅₀
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



Precautions

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25°C) 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

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.

