KAMIYA BIOMEDICAL COMPANY

Rat Gc-Globulin ELISA

For the quantitative determination of Gc-globulin in rat serum or plasma.

Cat. No. KT-481

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT
The **K-ASSAY®** Rat Gc-Globulin ELISA is an enzyme immunoassay for the quantitative determination of Gc-Globulin in rat serum or plasma. For research use only.

INTRODUCTION
Gc-globulin, also known as vitamin D-binding protein, is an acute phase protein that is synthesized in the liver and serves as an actin scavenger in blood. It has recently been identified as a potentially useful early marker of liver toxicity and is reportedly useful as an indicator of skeletal muscle injury.

PRINCIPLE
The **K-ASSAY®** Rat Gc-Globulin ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses an affinity purified anti-rat Gc-Globulin antibody for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated anti-rat Gc-Globulin antibody for detection. The test sample is diluted into actin containing diluent (converting free Gc-globulin to the actin complexed form) and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. This results in Gc-Globulin/actin complexes being 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, resulting 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 Gc-globulin is proportional to the optical density of the test sample.

COMPONENTS
- Anti-rat Gc-globulin antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 mL
- Calibrator (lyophilized)
- Actin (lyophilized), 3 vials
- 10X Diluent (25 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
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker with mixing speed of ~150 rpm
- A microtiter plate reader capable of measuring absorbance at 450 nm, with a bandwidth of 10 nm or less and an OD range of 0-4 OD
- Graph paper (PC graphing software is optional)

GENERAL INSTRUCTIONS
All reagents should be allowed to reach room temperature (18-25℃) before use.
Serum or plasma samples should be diluted ~10,000 fold with diluent in order to obtain values within the calibration range.

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

Add 5 mL of 10X diluent to one vial of lyophilized actin. Mix gently until the actin dissolves and then mix the 5 mL of actin containing 10X diluent with 45 mL of distilled or de-ionized water. The actin containing 1X diluent should be used within 2 hours of preparation.

CALIBRATOR PREPARATION

1. Add the volume of distilled or de-ionized water indicated on the lyophilized rat Gc-globulin calibrator vial label to the calibrator vial and mix gently until dissolved. This provides a 2 µg/mL stock.
2. Label 8 polypropylene microcentrifuge tubes as 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 ng/mL.
3. Dispense 437.5 µL of actin containing 1X diluent into the tube labeled 250 ng/mL and 250 µL of actin containing 1X diluent into the tubes labeled 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 ng/mL.
4. Prepare a 125 ng/mL calibrator by diluting and mixing 250 µL of reconstituted 250 ng/mL calibrator with 250 µL of diluent in the tube labeled 125 ng/mL.
5. Similarly prepare the 62.5, 31.25, 15.6, 7.8 and 3.9 ng/mL calibrators by serial dilution.

Please Note: The reconstituted calibrator should be aliquoted and frozen at -20°C after reconstitution if future use is intended.

SAMPLE PREPARATION

General Note: Our studies indicate that Gc-globulin is present in normal rat serum at a concentration of ~1 mg/mL. In order to obtain values within the range of the calibration curve we suggest that samples initially be diluted 10,000 fold in actin containing 1X diluent using the following procedure for each sample to be tested:

1. Dispense 198 µL and 297 µL of actin containing 1X diluent into separate tubes.
2. Pipette and mix 2 µL of the serum/plasma sample into the tube containing 198 µL of diluent. This provides a 100 fold diluted sample.
3. Mix 3 µL of the 100 fold diluted sample with the 297 µL of diluent in the second tube. This provides a 10,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µL of calibrators and 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. Wash and empty the microtiter wells 5 times with 1X wash solution. This may be performed using either a plate washer (400 µL/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
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 above.
9. Strike the wells sharply onto adsorbent paper or paper towels to remove all residual droplets.
10. Dispense 100 µL of TMB Reagent into each well.
11. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
12. 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 the optical density at 450 nm with a microtiter plate reader within 5 minutes.

CALCULATION OF RESULTS

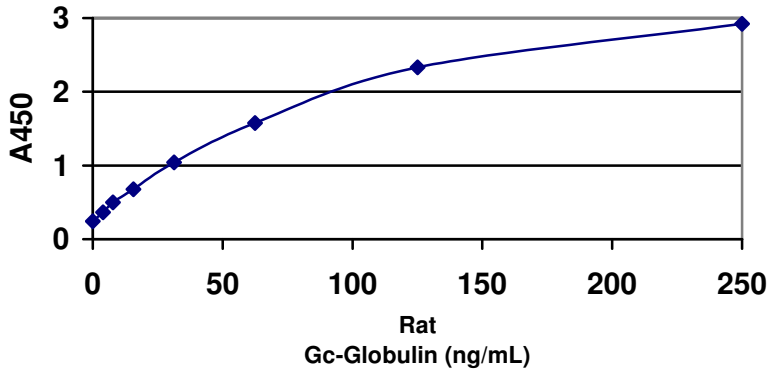
1. Calculate the average absorbance values (A_{450}) for each set of reference calibrators, and samples.
2. Construct a calibration curve by plotting the mean absorbance obtained from each reference calibrator against its concentration in ng/mL on linear graph paper, with absorbance values on the vertical or Y-axis and concentration on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of Gc-globulin in ng/mL from the calibration curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of Gc-globulin in the serum/plasma sample.
5. If available, PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside of the calibration curve when tested at a dilution of 10,000, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

A typical calibration curve with optical density reading at 450 nm on the Y axis against Gc-globulin 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.

Gc-globulin (ng/mL)	Absorbance (450 nm)
250	2.920
125	2.333
62.5	1.574
31.25	1.041
15.6	0.678
7.8	0.498
3.9	0.365
0	0.244

Typical Rat Gc-Globulin Calibration Curve



STORAGE

Lyophilized actin should be stored at or below -20℃. The remainder of the kit should be stored at 4℃ and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable until the expiration date provided that the components are stored as described above.

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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