KAMIYA BIOMEDICAL COMPANY

Guinea Pig Complement C3 ELISA

For the quantitative determination of Complement C3 in guinea pig biological samples

Cat. No. KT-338

For Research Use Only.

PRODUCT INFORMATION

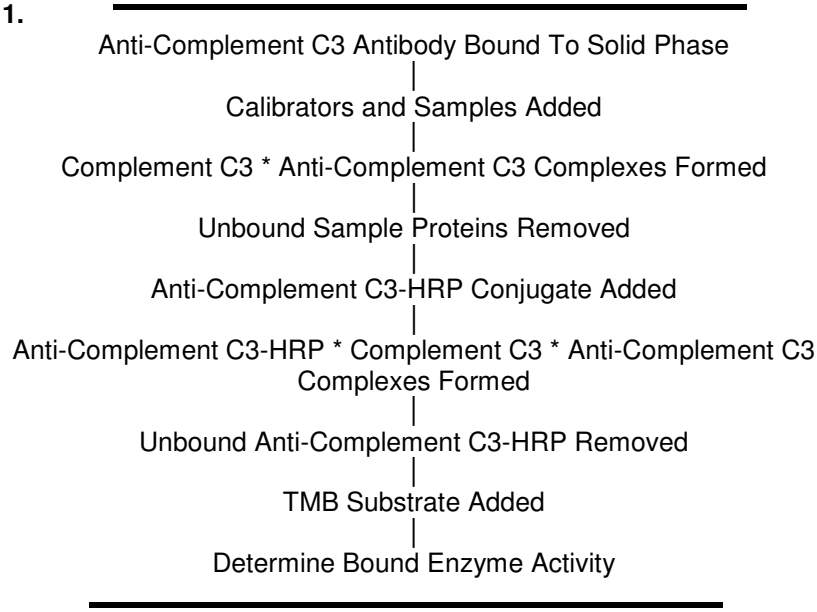
Guinea Pig Complement C3 ELISA
Cat. No. KT-338

INTENDED USE
The Guinea Pig Complement C3 ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of Complement C3 in Guinea Pig biological samples. For research use only.

INTRODUCTION
A number of serum proteins participate in acute inflammatory reactions. These include the complement, coagulation and kinin systems as well as a number of other proteins, known as acute phase proteins, which regulate acute inflammation. The complement system is a complex set of up to 20 different serum proteins. The most abundant and pivotal of the complement components is Complement C3, which has a molecular weight of about 187 kDa and consists of an alpha and beta chain.

PRINCIPLE
The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Complement C3 present in samples reacts with the anti-Complement C3 antibody, which has been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-Complement C3 antibody conjugated with horseradish peroxidase (HRP) is added. This HRP-conjugated antibody forms a complex with the previously bound Complement C3. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme is proportional to the concentration of Complement C3 in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Complement C3 in the test sample. The quantity of Complement C3 in the test sample can be interpolated from the calibration curve constructed from the calibrators and corrected for sample dilution.

Figure 1.



COMPONENTS
1. Diluent Concentrate
   One bottle containing 50 mL of a 5X concentrated diluent running buffer.

2. Wash Solution Concentrate
   One bottle containing 50 mL of a 20X concentrated wash solution.

3. Enzyme-Antibody Conjugate Concentrate
   One vial containing 150 µL of a 100X concentrated affinity-purified anti-guinea pig Complement C3 antibody conjugated with HRP in stabilizing buffer.

4. TMB Substrate Solution
   One vial containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.

5. Stop Solution
   One vial containing 12 mL of 0.3 M sulfuric acid. WARNING: Avoid contact with skin.

6. Microtiter Plate
   Twelve removable eight-well strips in well holder frame. Wells are coated with affinity-purified anti-guinea pig Complement C3.

7. Guinea Pig Complement C3 Calibrator
   One vial containing 100 µL with 0.326 mg/mL of guinea pig Complement C3.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microplate washer/aspirator
- Distilled or de-ionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
5. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
6. Other precautions:
   - Do not interchange kit components from different lots.
   - Do not use kit components beyond the expiration date.
   - Protect reagents from direct sunlight.
   - Do not pipette by mouth.
   - Do not eat, drink, smoke or apply cosmetics where reagents are used.
   - Avoid all contact with the reagents by using gloves.
   - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION

1. Diluent Concentrate
   The Diluent solution supplied is a 5X concentrate and must be diluted 1:5 with distilled or de-ionized water.

2. Wash Solution Concentrate
   The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Enzyme-Antibody Conjugate Concentrate
   Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL Enzyme-Antibody Conjugate to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. TMB Substrate Solution
   Ready to use as supplied.

5. Stop Solution
   Ready to use as supplied.

6. Microtiter Plate
   Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal. Take clear plastic tape, or parafilm, and cover tops of strips to avoid particulates from contaminating wells.

7. Guinea Pig Complement C3 Calibrator
   The Guinea Pig Complement C3 Calibrator should be aliquoted out and stored frozen. It is at a concentration of 0.326 mg/mL and needs to be diluted in 1X diluent immediately prior to use for each run (see chart below). Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator Volume added to 1X Diluent →	Volume of 1X Diluent
A	3,260	10 µL Guinea Pig Complement C3 Calibrator	990 µL
7	200	60 µL Calibrator A	918 µL
6	100	300 µL Calibrator 7	300 µL
5	50	300 µL Calibrator 6	300 µL
4	25	300 µL Calibrator 5	300 µL
3	12.5	300 µL Calibrator 4	300 µL
2	6.25	300 µL Calibrator 3	300 µL
1	3.125	300 µL Calibrator 2	300 µL
0			500 µL

STORAGE AND STABILITY

1. Complete Kit
   The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long term storage recommendations below for the Guinea Pig Complement C3 Calibrator.**

2. Diluent
   The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

3. Wash Solution
   The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (RT, 16-25°C) or at 4°C.

4. Enzyme-Antibody Conjugate
   Undiluted anti-Complement C3-HRP conjugate should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for up to 8 hours.

5. TMB Substrate Solution
   The TMB Substrate Solution should be stored at 4°C and is stable until the expiration date.

6. Stop Solution
   The Stop Solution should be stored at 4°C and is stable until the expiration date.

7. Microtiter Plate
   Anti-guinea pig Complement C3 coated wells are stable until the expiration date, and should be stored at 4°C in the sealed foil pouch with desiccant pack.

8. Guinea Pig Complement C3 Calibrator
   Aliquot Guinea Pig Complement C3 Calibrator and store them frozen, avoid repeated freeze-thaw cycles. For storage longer than 14 days, keep frozen until the expiration date. Storage for less than 14 days can be kept at 4°C. The working calibrator solutions should be prepared immediately prior to use and are stable for up to 8 hours.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture and the serum separated from the cells, after clot formation, by centrifugation. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Specimens may be shipped at room temperature and then stored refrigerated at 4°C if testing is to take place one week after collection. If testing is to take place later than one week, specimens should be stored at -20°C. Avoid repeated freeze-thaw cycles.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

ASSAY PROTOCOL

Dilution of Samples

The assay for quantification of Complement C3 in serum requires that each test sample be diluted before use. For a single step determination a dilution of serum at 1:10,000 is appropriate for most samples. For absolute quantification, samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

To prepare a 1:10,000 dilution of sample, transfer 5 µL of sample to 495 µL of 1X Diluent. This gives you a 1:100 dilution. Mix thoroughly. Next, dilute the 1:100 samples by transferring 5 µL to 495 µL of 1X Diluent. You now have a 1:10,000 dilution of your sample. Mix thoroughly at each stage.

Procedure

1. Bring all reagents to RT before use.

2. Pipette 100 µL of
   Calibrator 0 (0.0 ng/mL) in duplicate
   Calibrator 1 (3.125 ng/mL) in duplicate
   Calibrator 2 (6.25 ng/mL) in duplicate
   Calibrator 3 (12.5 ng/mL) in duplicate
   Calibrator 4 (25 ng/mL) in duplicate
   Calibrator 5 (50 ng/mL) in duplicate
   Calibrator 6 (100 ng/mL) in duplicate
   Calibrator 7 (200 ng/mL) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre designated wells.

4. Incubate the Microtiter Plate at 22°C (RT) for ten (10 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual Wash Solution. Repeat three times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (RT) for ten (10 ± 2) minutes. Keep plate covered and level during incubation.

8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 µL of TMB Substrate Solution into each well.

10. Incubate in the dark at RT for precisely five (5) minutes.

11. After five (5) minutes, add 100 µL of Stop Solution to each well.

12. Determine the absorbance at 450 nm of the contents of each well. Zero the plate reader to air.

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve, although a second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from calibration curve. Correct for sample dilution factor to arrive at Complement C3 concentration in original sample.

PERFORMANCE CHARACTERISTICS

In accord with good laboratory practice, the assays for specific Complement C3 require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, and accuracy of reagent and sample pipettings.

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