C3 (Guinea Pig) ELISA Kit

Catalog Number KA1925

96 assays

Version: 02

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Intended Use

The C3 (Guinea Pig) ELISA Kit is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the measuring C3 in biological samples of Guinea Pig.

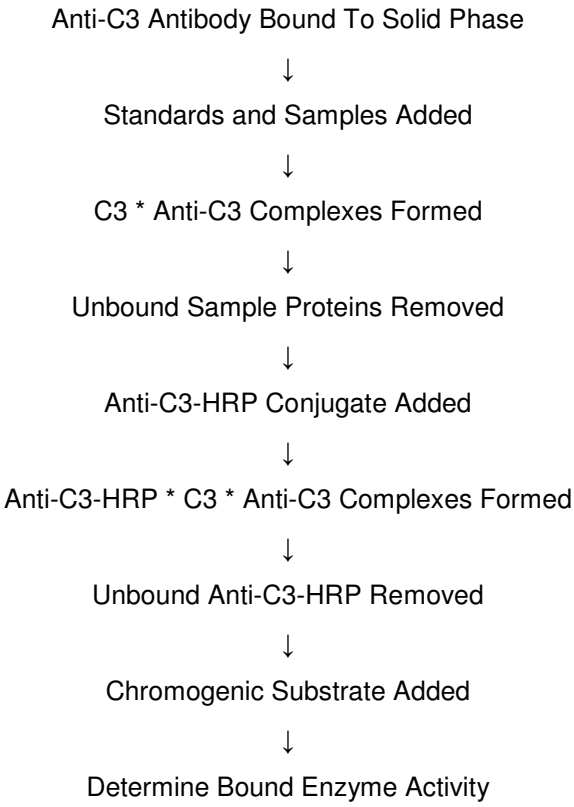
Background

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 of the Assay

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-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 C3 in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of C3 in the test sample. The quantity of C3 in the test sample can be interpolated from the calibration curve constructed from the standards, and corrected for sample dilution.

Figure 1.


Anti-C3 Antibody Bound To Solid Phase
↓
Standards and Samples Added
↓
C3 * Anti-C3 Complexes Formed
↓
Unbound Sample Proteins Removed
↓
Anti-C3-HRP Conjugate Added
↓
Anti-C3-HRP * C3 * Anti-C3 Complexes Formed
↓
Unbound Anti-C3-HRP Removed
↓
Chromogenic Substrate Added
↓
Determine Bound Enzyme Activity

General Information

Materials Supplied

List of component

Component	Amount
Diluent Concentrate (Running Buffer): One bottle containing a 5X concentrated diluent running buffer.	50 mL
Wash Solution Concentrate: One bottle containing a 20X concentrated wash solution.	50 mL
Enzyme-Antibody Conjugate 100X: One vial containing affinity purified anti- Guinea Pig C3 antibody conjugated with horseradish peroxidase in a stabilizing buffer.	150 µL
Chromogen-Substrate Solution: One vial containing 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.	12 mL
Stop Solution: One vial containing 0.3 M sulfuric acid. WARNING: Avoid contact with skin.	12 mL
Anti-Guinea Pig C3 ELISA micro plate: Twelve removable eight (8) well strips in well holder frame. Each well is coated with affinity purified anti-Guinea Pig C3	96 wells
Guinea Pig C3 Standards: One vial containing Guinea Pig C3 Calibrator	1 vial

Storage Instruction

The expiration date for the package is stated on the box label.

1. 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 4-8℃.
2. 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 (16-25℃) or at 4-8℃.
3. Enzyme-Antibody Conjugate
   Undiluted horseradish peroxidase anti-C3 conjugate should be stored at 4-8℃ and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.
4. Chromogenic Substrate Solution
   The Substrate Solution should be stored at 4-8℃ and is stable until the expiration date.
5. Stop Solution
   The Stop Solution should be stored at 4-8℃ and is stable until the expiration date.
6. Anti-Guinea Pig C3 ELISA micro plate
   Anti-Guinea Pig C3 coated wells are stable until the expiration date, and should be stored at 4-8℃ in the sealed foil pouch with desiccant pack.

7. Guinea Pig C3 Calibrator

   Aliquot Guinea Pig 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℃. The working calibrator solutions should be prepared immediately prior to use and are stable for up to 8 hours.

Materials Required but Not Supplied

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

Precautions for Use

- ✓ Precaution

  For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

- ✓ Additives and Preservatives

  No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

- ✓ Known interfering substances

  Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

- ✓ Limitation of the procedure

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
- 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.

Assay Protocol

Reagent Preparation

- ✓ Diluent Concentrate

  The Diluent solution supplied is a 5X concentrate and must be diluted 1/5 (1 part buffer concentrate, 4 parts dH_2O) with distilled or deionized water.
- ✓ Wash Solution Concentrate

  The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH_2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35℃ before dilution can dissolve crystals.
- ✓ Enzyme-Antibody Conjugate

  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.
- ✓ Chromogen-Substrate Solution

  Ready to use as supplied.
- ✓ Stop Solution

  Ready to use as supplied.
- ✓ Anti-Guinea Pig C3 ELISA Micro 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.
- ✓ Guinea Pig C3 Standards

  The Guinea Pig C3 Calibrator should be aliquoted out and stored frozen. It is at a concentration of 326 µg/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.

Standard	ng/mL	Volume added to 1X Diluent	Volume of 1X Diluent
A	3200	5 µL Guinea Pig C3 Calibrator	495 µL
7	200	60 µL standard A	918 µL
6	100	300 µL standard 7	300 µL
5	50	300 µL standard 6	300 µL
4	25	300 µL standard 5	300 µL
3	12.5	300 µL standard 4	300 µL
2	6.25	300 µL standard 3	300 µL
1	3.125	300 µL standard 2	300 µL
0			600 µL

Sample Preparation

✓ Specimen Collection and Handling

Blood should be collected by venipuncture. The serum should be 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 2-8℃ if testing is to take place within one week after collection. If testing is to take place later than one week, specimens should be stored at -20℃. Avoid repeated freeze-thaw cycles.

✓ Dilution of Samples

The assay for quantification of C3 in samples requires that each test sample be diluted before use. For a single step determination a dilution of 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. 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.

Assay Procedure

1. Bring all reagents to room temperature before use.
2. Pipette 100 µL of
   Standard 0 (0.0 ng/mL) in duplicate
   Standard 1 (3.125 ng/mL) in duplicate
   Standard 2 (6.25 ng/mL) in duplicate
   Standard 3 (12.5 ng/mL) in duplicate
   Standard 4 (25 ng/mL) in duplicate
   Standard 5 (50 ng/mL) in duplicate
   Standard 6 (100 ng/mL) in duplicate
   Standard 7 (200 ng/mL) in duplicate
3. Pipette 100 µL of sample (in duplicate) into pre designated cells.
4. Incubate the Micro titer Plate at room temperature 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 wash buffer, 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 buffer. Repeat 3 times for a total of four washes.

7. Pipette 100 µL of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for ten (10 ± 2) minutes. Keep plate covered in the dark and level during incubation.
8. Wash and blot the wells as described in Steps 5/6.
9. Pipette 100 µL of TMB Substrate Solution into each well.
10. Incubate in the dark at room temperature for precisely five (5) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance at 450 nm of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

✓ Stability of the final reaction mixture

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.

Data Analysis

Calculation of Results

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the standards construct a standard curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the C3 concentration in original samples.

Resources

Plate Layout

	A	B	C	D	E	F	G	H
12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								