Canine Complement Factor 3 ELISA Assay Kit

Catalog Number: CC341-K01

For Research Use Only. Not for use in diagnostic procedures.

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INTENDED USE

The C3 test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring C3 in dog biological samples. If the ELISA is to be used outside the intended use, the user may need to optimize for said use.

For further information about this kit, its application, or the procedures in this insert, please contact the Technical Service Team at Eagle Biosciences, Inc at www.EagleBio.com or at 866-411-8023.

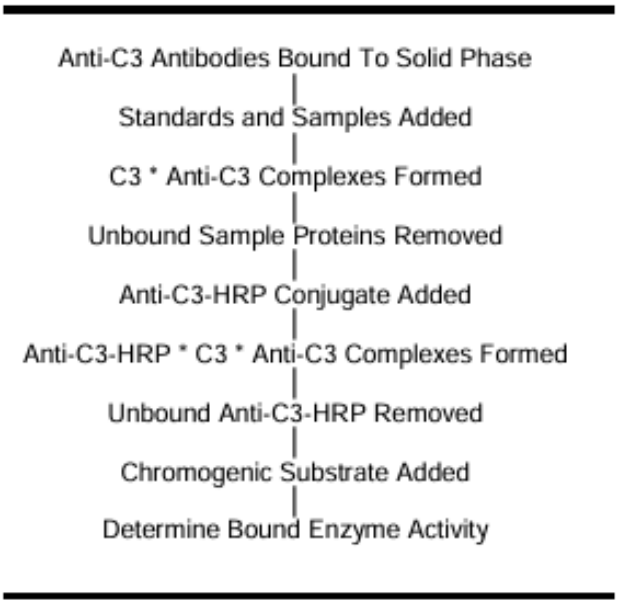
ASSAY BACKGROUND

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, that regulate acute inflammation.

The complement system is a complex set of up to 20 serum proteins. The most abundant and pivotal of the complement components is C3, which has a molecular weight of about 187 kD 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 factor 3 (C3) present in samples reacts with the anti-C3 antibodies, which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-C3 antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound 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 varies directly with 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 standard curve constructed from the standards, and corrected for sample dilution



LIMITATION OF THE PROCEDURE

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. Factors that might affect the performance of the assay include instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature. Do not mix or substitute reagents with those from other lots or sources.

ELISA Micro Plate	One plate of 12 Removable 8 well strips, antibody coated.	Ready to use as supplied.	4-8ºC, in sealed foil bag with desiccant	With proper storage the plate strips are stable until expiration
Enzyme Conjugated Detection Antibody	One vial of 150 µL of 100x Horseradish Peroxidase Conjugated antibody in a stabilizing buffer	Dilute 1/100 immediately prior to use.	4-8ºC in the dark	The working conjugate solution should be diluted immediately prior to use. The 100X conjugate is stable until the expiration date
Calibrator	One vial of calibrator	Refer to the Certificate of Analysis (CoA)	4-8ºC for lyophilized calibrator. Aliquoted and frozen if re-constituted. Avoid multiple freeze-thaw cycles.	The working standard solutions should be prepared immediately prior to use.
Diluent concentrate	One 10 mL bottle of 5X diluent buffer	Dilute 1/5 to make 1X working solution	4-8ºC for both 1X working solution and 5X concentrate	The 1X working solution is stable for at least one week from the date of preparation. The 5X concentrate is stable until the expiration date.
Wash Solution Concentrate	One 50 mL bottle of 20X working solution	Dilute 1./20 to make 1X working solution.	4-8ºC for both 1X working solution and 20X working solution	The 1X working solution is stable for at least one week from the date of preparation. The 20X concentration is stable until the expiration date.
Chromogen-Substrate Solution	One bottle of 12 mL 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3	Ready to use as supplied	4-8ºC in the dark	Protect from light. The Substrate Solution is stable until the expiration date.
STOP Solution **WARNING: Avoid contact with skin**	One 12 mL bottle of 0.3M sulfuric acid.	Ready to use as supplied	4-8ºC	The Stop Solution is stable until the expiration date.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 µL to 100 µL) for making and dispensing dilutions
- Test tubes
- Squirt bottle or Microtitre washer/aspirator
- Distilled or Deionized H2O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Centrifuge for sample collection
- Anticoagulant for plasma collection
- Timer

DILUTION OF SAMPLES

The assay requires that each test sample be diluted before use. All samples should be assayed in duplicate each time the assay is performed. The recommended dilutions are only suggestions. Dilutions should be based on the expected concentration of the unknown sample such that the diluted sample falls within the dynamic range of the standard curve. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

Serum samples – Recommended starting dilution is 1/40,000. To prepare a 1/40,000 dilution of a sample, transfer 5 µL of sample to 995 µL of 1X diluent. This gives you a 1/200 dilution. Next, dilute the 1/200 by transferring 5 µL into 995 µL of 1X diluent. This gives you a 1/40,000 dilution. Mix thoroughly each stage.

Plasma samples – Recommended starting dilution is 1/40,000. To prepare a 1/40,000 dilution of a sample, transfer 5 µL of sample to 995 µL of 1X diluent. This gives you a 1/200 dilution. Next, dilute the 1/200 by transferring 5 µL into 995 µL of 1X diluent. This gives you a 1/40,000 dilution. Mix thoroughly each stage.

REAGENT PREPARATION

Bring all reagents to room temperature (16ºC to 25ºC) before use.

Diluent Concentrate - The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH2O).

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 dH2O). Crystal formation in the concentrate may occur when storage temperatures are low. Warming of the concentrate to 30-35ºC before dilution can dissolve crystals.

Enzyme-Antibody Conjugate - Calculate the required amount of working conjugate solution for each microtitre 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. Dilute immediately before use and protect from light. Mix uniformly, but gently. Avoid foaming.

Pre-coated ELISA Micro Plate - Ready to use as supplied. Unseal foil 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 along with desiccant.

Dog C3 Calibrator – Prepare according to the lot specific Certificate of Analysis.

ASSAY PROCEDURE

1. All samples and standards should be assayed in duplicates.
2. The Standards and the test sample(s) should be loaded into the ELISA wells as quickly as

   possible to avoid a shift in OD readings. Using a multichannel pipette would reduce this occurrence.

   Pipette 100 µL of

   Standard 0 (0.0 ng/mL) in duplicate
   Standard 1 (6.25 ng/mL) in duplicate
   Standard 2 (12.50 ng/mL) in duplicate
   Standard 3 (25 ng/mL) in duplicate
   Standard 4 (50 ng/mL) in duplicate
   Standard 5 (100 ng/mL) in duplicate
   Standard 6 (200 ng/mL) in duplicate

3. Pipette 100 µL of sample (in duplicate) into pre designated wells.
4. Incubate the micro titer plate at room temperature for twenty (20 ± 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 then 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 twenty (20 ± 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 ten (10) minutes.
11. After ten minutes, add 100 µL of Stop Solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well within 30 minutes. Calibrate the plate reader to manufacturer's specifications

CALCULATION OF RESULTS

1. Subtract the average background value (Average absorbance reading of Standard zero) from the test values for each sample.
2. Average the duplicate readings for each standard and use the results to construct a Standard Curve. Construct the standard curve by reducing the data using computer software capable of generating a four parameter logistic curve fit. A second order polynomial (quadratic) or other curve fits may also be used; however, they will be a less precise fit of the data.
3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the C3 concentration in original samples.

WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.