



Complement Factor 3 (Mouse) ELISA

For the quantitative determination of complement factor 3 (C3)
in serum or plasma of mice

Please read carefully due to Critical Changes, e.g., Calibrator concentration.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	41-CO3MS-E01
Size:	96 wells
Version:	2 L9.0 - ALPCO 11/17/2009

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INTENDED USE

The Complement factor 3 (C3) test kits are highly sensitive two-site enzyme linked immunoassays (ELISA) for measuring C3 in serum and plasma of mice.

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 serum proteins. The most abundant and pivotal of the complement components is C3, which has a molecular weight of approximately 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 C3 present in the samples reacts with the anti-C3 antibodies which have been adsorbed to the surface of polystyrene microtiter 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; 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.

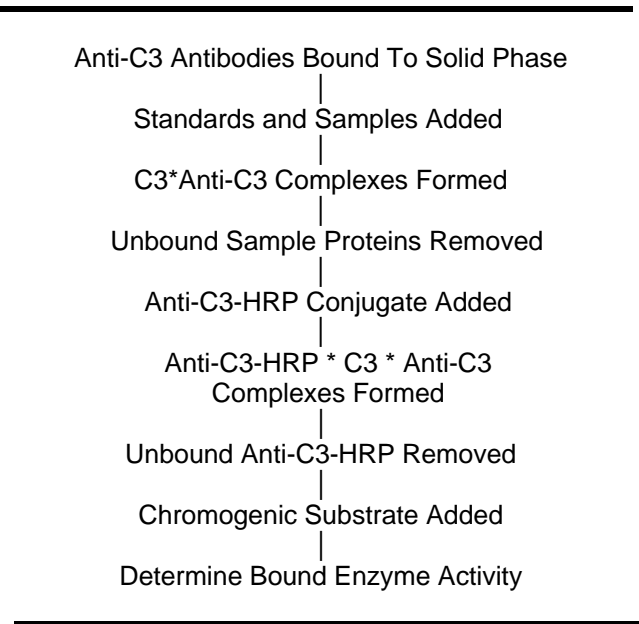


Figure 1.

REAGENTS (Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE (assay buffer)

One bottle containing 50 ml of a 5X concentrated Diluent (assay buffer).

2. WASH SOLUTION CONCENTRATE

One bottle containing 50 ml of a 20X concentrated Wash solution.

3. ENZYME ANTIBODY CONJUGATE 100X

One vial containing 150 µl of affinity purified anti-C3 antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN SUBSTRATE SOLUTION

One vial containing 12 ml of 3,3',5,5'-tetramethylbenzidine (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. ANTI-C3 MICROPLATE

Twelve removable eight (8) well microplate strips in well holder frame. Each well is coated with affinity purified anti-C3.

7. C3 CALIBRATOR

One vial containing a lyophilized C3 Calibrator.

8. REFERENCE SERUM

One vial containing a C3 Reference Serum. (See Product Profile sheet enclosed with kit).

REAGENT PREPARATION

1. DILUENT CONCENTRATE

The Diluent solution supplied is a 5X concentrate and must be diluted 1/5 with deionized water (1 part Diluent concentrate, 4 parts deionized water).

2. WASH SOLUTION CONCENTRATE

The Wash solution supplied is a 20X concentrate and must be diluted 1/20 with deionized water (1 part Wash concentrate, 19 parts deionized 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

Prepare the required amount of working Conjugate solution for each microplate strip by adding 10 µl of Enzyme Antibody Conjugate to 990 µl of 1X Diluent for each strip to be used. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN SUBSTRATE SOLUTION

Ready to use as supplied.

5. STOP SOLUTION

Ready to use as supplied.

6. ANTI-C3 MICROPLATE

Ready to use as supplied. Unseal Microplate pouch and remove plate from pouch. Remove all strips and wells that **will not** be used from the well holder frame, place back in pouch along with desiccant pack, and reseal.

7. C3 CALIBRATOR

Add 1.0 ml of deionized water to the C3 Calibrator and mix gently until dissolved. The Calibrator is now at a concentration of 2.82 µg/ml (**the reconstituted Calibrator should be frozen in aliquots if future use is intended**). **C3 Standards need to be prepared immediately prior to use (see chart below)**. Mix well between each step. Avoid foaming.

Standards	ng/ml	Volume Added to 1X Diluent	Volume of 1X Diluent
7	200	40 µl of C3 Calibrator	524 µl
6	100	300 µl of Standard 7	300 µl
5	50	300 µl of Standard 6	300 µl
4	25	300 µl of Standard 5	300 µl
3	12.5	300 µl of Standard 4	300 µl
2	6.25	300 µl of Standard 3	300 µl
1	3.125	300 µl of Standard 2	300 µl
0	0		500 µl

8. REFERENCE SERUM

The Reference Serum should be diluted as appropriate to fit within the standard curve range.

STORAGE AND STABILITY

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

1. DILUENT

The 5X Diluent concentrate is stable until the expiry 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°C.

2. WASH SOLUTION

The 20X Wash solution concentrate is stable until the expiry 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°C) or at 4-8°C.

3. ENZYME ANTIBODY CONJUGATE

Undiluted horseradish peroxidase anti-C3 conjugate should be stored at 4-8°C and **diluted immediately prior to use**. The working conjugate solution is stable for up to 8 hours.

4. CHROMOGEN SUBSTRATE SOLUTION

The Chromogen Substrate solution should be stored at 4-8°C and is stable until the expiry date.

5. STOP SOLUTION

The Stop solution should be stored at 4-8°C and is stable until the expiry date.

6. ANTI-C3 MICROPLATE

Anti-C3 coated wells are stable until the expiry date and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

7. C3 CALIBRATOR

The lyophilized C3 Calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted Calibrator should be stored frozen in aliquots (avoid multiple freeze-thaw cycles). The working Standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

8. REFERENCE SERUM

The Reference Serum is stable until the expiry date.

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis; excessive hemolysis can impact the results. Assay immediately or store samples in aliquots at -20°C. Avoid repeated freeze-thaw cycles.

1. Precautions

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

2. Additives and Preservatives

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

3. Known Interfering Substances

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

MATERIALS PROVIDED - See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (4 µl - 1 ml) for making and dispensing dilutions
- Test tubes
- Microplate washer/aspirator
- Deionized or distilled water
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer
- Centrifuge
- Anticoagulant – for collection of plasma samples

ASSAY PROTOCOL

DILUTION OF SAMPLES AND REFERENCE SERUM

The assay for quantification of C3 requires that the samples and reference serum be diluted before use. For a single step determination a dilution of 1/50,000 is appropriate for most plasma/serum samples. A lesser or greater dilution might be required for absolute quantification of samples yielding results outside the range of the standard curve. **If unsure of sample level, it is highly recommended to perform a serial dilution with one or two representative samples before running the entire plate.**

1. To prepare a 1/50,000 dilution of sample, transfer 5 µl of sample to 995 µl of 1X Diluent. This yields a 1/200 dilution. Next, dilute the 1/200 sample by transferring 4 µl to 996 µl of 1X Diluent. You now have a 1/50,000 dilution of your sample. Mix thoroughly at each stage.

PROCEDURE

1. **Bring all reagents to room temperature before use.**

2. Pipette 100 µl of

- Standard 0 (0 ng/ml) in duplicate
- Standard 1 (6.25 ng/ml) in duplicate
- Standard 2 (12.5 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 the prediluted samples and Reference serum into the predesignated wells (in duplicate).

4. Incubate the microplate 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 1X Wash solution, invert the plate, and then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual solution. Repeat three times for a total of four washes.
7. Pipette 100 μ l of appropriately diluted Enzyme Antibody Conjugate to each well. Incubate at room temperature for twenty (20 +/- 2) minutes. Keep plate covered, level, and in the dark during the incubation.
8. Wash and blot the wells as described in Steps 5 and 6.
9. Pipette 100 μ l of Chromogen Substrate solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 μ l of Stop solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to air.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to two 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 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 fit may also be used.
3. Interpolate test sample values from the standard curve. Correct for sera dilution factor to arrive at the C3 concentration in the original sample.

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 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 deionized water; and accuracy of reagent and sample pipettings, washing technique, and incubation times/temperatures.
3. Do not mix or substitute reagents with those from other lots or sources.