PERFORMANCE CHARACTERISTICS (continued)

Sample Recovery
High and low concentrations of purified mouse C3 were spiked into each of 3 serum samples. Observed assay values compared to expected values ranged from 88 to 115%, indicating accurate quantification of C3 in mouse serum.

Sample	Expected ng/ml	Observed ng/ml	Observed/ Expected
High C3 Spike		29.5	
+ Mouse D, 28.1 ng/ml	68.1	74.8	**110 %**
+ Mouse E, 38.0 ng/ml	78.0	81.1	**104 %**
+ Mouse F, 13.6 ng/ml	53.6	60.1	**112 %**
Low C3 Spike		9.0	
+ Mouse D, 28.1 ng/ml	37.1	32.5	**88 %**
+ Mouse E, 38.0 ng/ml	47.0	49.1	**104 %**
+ Mouse F, 13.6 ng/ml	22.6	26.1	**115 %**

ELISA Kit Components	Amount	Part No.
Anti-Mouse C3 Microwell Strip Plate	8-well strips (12)	6271
Mouse C3 Positive Control	0.65 ml	6272
Mouse C3 Standard 12.5 ng/ml	0.65 ml	6273B
Mouse C3 Standard 25 ng/ml	0.65 ml	6273C
Mouse C3 Standard 50 ng/ml	0.65 ml	6273D
Mouse C3 Standard 100 ng/ml	0.65 ml	6273E
Mouse C3 Standard 200 ng/ml	0.65 ml	6273F
Anti-Mouse C3 HRP Conjugate (100X)	0.15 ml	6274
Sample Diluent Concentrate (20X)	10 ml	SD-20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-6270

For more details please consult our web site (www.4adi.com) or contact us by email (service@4adi.com).

Instruction Manual No. M-6270

Mouse C3

ELISA Kit Cat. No. 6270

For Quantitative Determination of Mouse Complement 3 in Serum

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

INTENDED USE

The Mouse C3 ELISA Kit is an in vitro immunoassay for the quantification of C3 circulating in serum or in other appropriately qualified samples from tissue fluids (e.g., saliva, mucosa), or in cultures of mouse cells.

RESEARCH USE OF THE TEST

Complement C3 is the central component in the complex cascade of the complement system, the natural defense mechanism that combines innate and adaptive immunity to protect the body from infection and cancer. C3 is the most abundant complement protein in serum, and is the common intersection of all 3 pathways that have been elucidated for initiation of the complement cascade: Classical, Alternative and Lectin. C3 promotes phagocytosis, supports local inflammatory responses against pathogens, and instructs the adaptive immune response to select appropriate antigens for antibody production.

Increased levels of circulating C3 are linked to acute inflammatory disease and tissue inflammation. Decreased levels are associated with autoimmune disease (e.g., systemic lupus erythematosus), bacteremia, tissue injury and chronic hepatitis, among others. Additionally, C3 has been shown to predict development of cardiovascular disease, and binds to fibrin and fibrinogen as a component of blood clots.

Quantification of mouse complement C3 is central to current research using mouse models aimed at elucidating the components and pathways of immune regulation, and for understanding and controlling C3 related diseases.

PRINCIPLE OF THE TEST

The Mouse C3 ELISA kit is based on the binding of mouse C3 in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of C3 present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of C3 in samples and control is calculated from a curve of standards containing known concentrations of C3.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the label. Stabilities of the working solutions are indicated under Reagent Preparation.

PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit have been shown by immunoelectrophoresis and ELISA to react specifically with C3, and have essentially no reactivity with any other mouse serum proteins.

Serum from the following species showed no significant reactivity at 1:400 dilution: human, rat, hamster, guinea pig, bovine, pig, horse, sheep, goat, dog, cat, rabbit or chicken; also 10% neonatal bovine serum.

Normal Range

Assay of C3 in stored sera from fourteen (14) individual Swiss mice ranged from 0.96 to 2.85 mg/ml (median = 1.28mg/ml). Total C3 varies with sex, age and strain. Each laboratory should determine expected values of its own testing population.

Precision

Samples containing low, medium and high concentrations of C3, representing 3 different sera, were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficient of variations were calculated for the concentrations using a point-to-point curve-fitting program.

C3 concentrations were measured with very good within-assay (2.3 to 6.9 %CV) and between-assay (7.7 to 8.3 %CV) reproducibility.

Sample	C3 ng/ml	Intra-assay %CV	Inter-assay %CV
Mouse A	23.0	**6.9**	**7.9**
Mouse B	49.9	**2.3**	**7.7**
Mouse C	102.7	**5.7**	**8.3**

Linearity of Dilution

Four (4) individual and one (1) pooled stored sera were diluted to 2 levels for testing, and concordance of the assay values were compared. The mean recovery ranged from 95 to 99%, demonstrating linear dilution and equivalent quantification across the standard range.

Sample	Dilution	Assay Value ng/ml	Serum Value mg/ml	Concordance
Mouse M1	1:20k 1:160k	57.5 7.4	1.15 1.18	**99 %**
Mouse M2	1:40k 1:160k	23.6 6.1	0.94 0.98	**98 %**
Mouse F1	1:40k 1:160k	64.5 16.2	2.58 2.59	**99 %**
Mouse F2	1:20k 1:160k	98.0 12.4	1.96 1.98	**99 %**
Mouse Pool	1:25k 1:125k	69.0 15.3	1.72 1.91	**95 %**

Continued on Page 7.

CALCULATION OF RESULTS

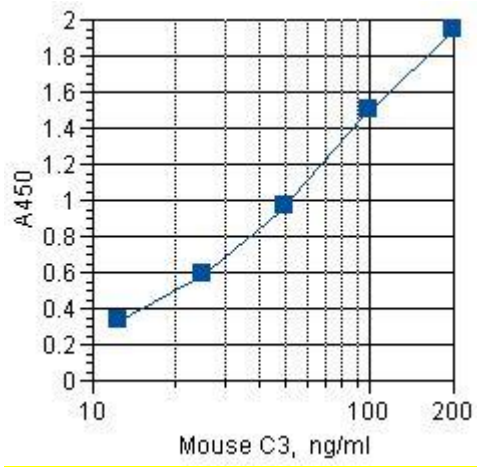
1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, C3 concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of C3 (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The C3 concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 200 ng/ml standard should be further diluted and re-assayed.

TYPICAL RESULTS

The following data are for illustration purposes only. A complete standard curve should be run in every assay to determine sample values.

Wells	Standards, Control & Samples	A450 nm	C3 ng/ml
A1, A2	**Negative Diluent Control**	0.04	0
B1, B2	12.5 ng/ml **Standard**	0.33	12.5
C1, C2	25 ng/ml **Standard**	0.59	25
D1, D2	50 ng/ml **Standard**	0.97	50
E1, E2	100 ng/ml **Standard**	1.50	100
F1, F2	200 ng/ml **Standard**	1.94	200
G1, G2	**Positive Serum Control** [Value: 35 - 65 ng/ml]	0.96	49
H1, H2	**Sample** [Diluted 1:40k]	0.64	27
	Calculated: 40k-fold dilution x 27 ng/ml = **1.08** mg/ml in serum		

A typical assay Standard Curve (do not use for calculating sample values)



KIT CONTENTS

To Be Reconstituted: Store as indicated.

Component	Instructions for Use
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at RT until kit is used entirely.
Anti-Mouse C3 - HRP Conjugate Concentrate (100x) Part No. 6274, 0.15ml	Peroxidase conjugated anti-mouse C3 in buffer with protein, detergents and ProClin 300 as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return concentrate to 2-8ºC storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
Anti-Mouse C3 Microwell Strip Plate	6271	8-well strips (12)	Coated with purified anti-Mouse C3 antibodies.
Mouse C3 Standards			
12.5 ng/ml 25 ng/ml 50 ng/ml 100 ng/ml 200 ng/ml	6273B 6273C 6273D 6273E 6273F	0.65 ml 0.65 ml 0.65 ml 0.65 ml 0.65 ml	Five (5) vials, each containing mouse serum with calibrated C3 concentrations; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
Positive Control [C3] range on label	6272	0.65 ml	Mouse serum with stated C3 concentration range; diluted in buffer with protein, detergents and ProClin 300 as stabilizers.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	1% sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-mouse C3-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera are not assayed immediately , store refrigerated for up to 2 weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.
The use of plasma has not been investigated, but should be a suitable specimen for assay.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Controls, Sample Diluent, and Anti-mouse C3-HRP contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.
MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Sample Controls A Positive Serum Control is provided with the kit, assigned with an C3 concentration value range. Recovery in this range is an indicator of proper assay performance. Each lab should also assay internal control samples, which represent the lab's expected sample population and that are maintained stabilized. A Negative Diluent Control should also be run.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-continuously increasing or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. A Negative Diluent Control should be of lower signal than the lowest standard. Do not rely on results generated from an assay with these issues.

ASSAY PROCEDURE

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

DILUTE Serum Samples in Working Sample Diluent. Dilutions of about 25k-fold are appropriate for most normal mouse sera. For accuracy, three dilution steps are recommended, as follows:

1) 10ul serum + 390ul diluent = [1:40],
2) 20ul [1:40] + 480ul diluent = [1:1k],
3) 20ul [1:1k] + 480ul diluent = **1:25k**

DO NOT diluted the Standards or Control.

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. Set-up

- Determine the number of wells for the assay run. Duplicates are recommended, including 10 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes before sample addition.
- Aspirate the liquid and pat dry on a paper towel.

2. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of standards, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

3. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-mouse C3-HRP Conjugate to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

4. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

5. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

6. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.