Fast Mouse Complement C3d ELISA

For the quantitation of mouse C3d concentrations in cell culture supernatants, serum, and plasma.

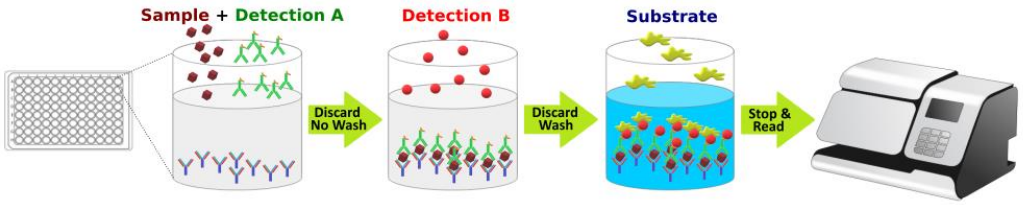
INTRODUCTION

Complement is a system of plasma proteins that interacts with pathogens to mark them for destruction by phagocytes. It is an essential component of our innate immunity, both for the protection against infections and for proper handling of dying cells, and contributing to tissue injury and inflammatory responses. Complement C3d, is the final degradation product of the third component of complement (C3). When conjugated to an antigen, C3d enhances immune responses to the fused antigen. Therefore, this molecule has been used as an adjuvant to enhance the immune responses to various foreign and self-proteins.

The Fast Mouse C3d ELISA is a solid phase ELISA designed to measure mouse C3d levels in cell culture supernatants, serum, and plasma. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated multiple steps in traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 3 hours, not need 4-5 hours (Fig. 1). The detection range is from 1 to 250 ng/mL. The levels of mouse C3d samples are parallel to the standard curves obtained using the kit standards linearly. These results indicate that this kit can be used to determine relative mass values for natural mouse C3d protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A polyclonal antibody specific for mouse C3d was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 2 hours. Then, just aspirate each well, no wash, directly add Streptavidin-HRP, incubate the complex. Following a wash to remove any unbound antibody and samples, an **ultra-sensitive TMB substrate solution** is added to the wells for color develops. The color intensity is in proportion to the amount of C3d bound in the initial step. The intensity of the color is measured by plate read at 450 nm.



KIT CONTENT AND STORAGE CONDITIONS

PART	PART#	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED
Mouse C3d Microplate	TBS3022A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for mouse C3d.	The unused wells can be stored in the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Mouse C3d Standard	TBS3022B	30 µl of Recombinant mouse C3d protein (10 µg/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost the freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3022C	2.1 ml of mouse C3d antibody.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3022D	120 µl of Streptavidin-HRP	
Assay Diluent	TBS3022E	25 ml of a buffered protein base with preservatives.	
10x Wash Buffer	TBS3000W	12 ml of concentrated solution (10x).	
TMB Substrate	TBS3000T	12 ml of ultra-sensitive TMB substrate.	
Stop Solution	TBS3000S	6 ml of 2 N sulfuric acid.	

Store the unopened kit at 2-8 ℃. Do not use past kit expiration date.
The kit contains sufficient materials to run an ELISA on one 96 well plate.

PRECAUTIONS

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

REAGENT PREPARATION

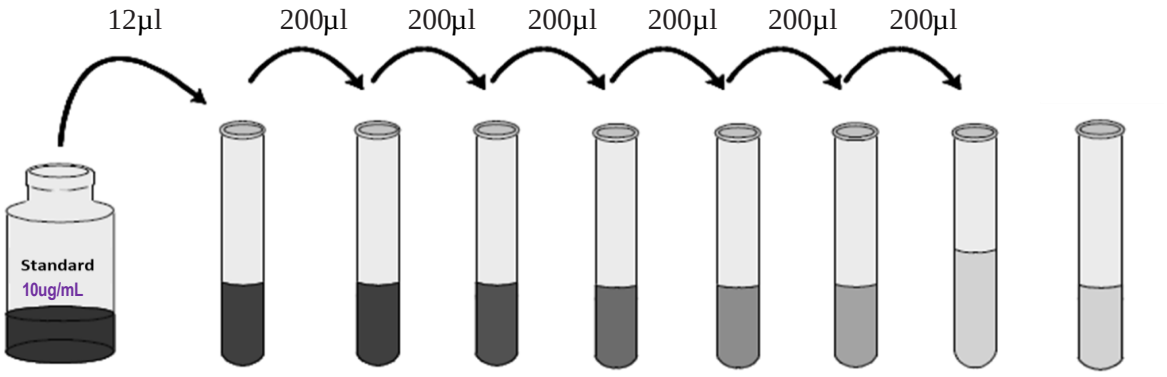
Bring all reagents to room temperature before use.

Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.).

Mouse C3D Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 468 µL of 1x Assay Diluent into tube #1, and 300 µL into tubes #2 to #8 **as diagram below.**
2. Add 12 µL of the Mouse C3D Standard stock solution (10 µg/mL) by dilution of 40 times to tube #1 and mix.
3. Make 2.5x serial dilutions of the standard using the 250 ng/mL standard solution from tube #2 through #7 with sequential transfer of 200 µL to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 250, 100, 40, 16, 6.4, 2.56 and 1.024 ng/mL. Tube# 8 is Standard 0.

Fig. 2 Diagram for Mouse C3D standard preparation



Standard	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	468	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	12	200	200	200	200	200	200	0
Final Conc. (ng/ml)	250	100	40	16	6.4	2.56	1.024	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Add 80 µL of standard, or sample, or control to the indicated well.
2. Add 20 µL of **Detection A** to the above standard and sample of each well, thoroughly mix. Cover with the adhesive sealer. Incubate at **RT for 2 hours.**
3. Aspirate each well (***no wash***). Invert the plate and blot it against clean paper towels.
4. Add 100 µL of **Detection B** to each well. Incubate at **RT for 1 hour.**
5. Aspirate each well, and wash for 3 times (3 minutes/each) by filling each well with 200 µL Wash Buffer (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of **TMB Substrate** to each well. Incubate **at RT for 10-20min** (*Protect from light*). The color becomes blue. If the color is light, the incubation time can be longer.
7. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
8. Determine the optical density of each well within 5 minutes, using a microplate reader at 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample subtract the average zero standard optical density (O.D.).

Create a standard curve using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse C3d concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

TYPICAL DATA

This standard curve(R^2=0.998) is provided for demonstration only. A standard curve should be generated for each set of samples assayed. Fig. 3 is an example of typical data.

SENSITIVITY

The minimum detectable dose (MOD) of mouse C3d is typically 1 ng/ml.

The Intra-assay CV and the Inter-assay CV is < 10%.

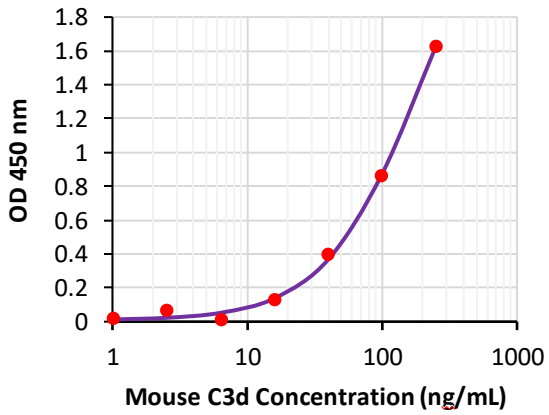
SPECIFICITY

This assay recognizes natural and recombinant mouse C3d.
No cross reaction: Human C3d.

RELATIVE PRODUCTS

TBS3030	Fast Mouse IL-1β ELISA
TBS3032	Fast Mouse IL-4 ELISA
TBS3040	Fast Mouse IL-6 ELISA
TBS3044	Fast Mouse IL-10 ELISA
TBS3047	Fast Mouse IL-12 p70 ELISA
TBS3049	Fast Mouse IL-13 ELISA
TBS3050	Fast Mouse TNF-α ELISA
TBS3060	Fast Mouse KC ELISA
TBS3070	Fast Mouse NGF ELISA
TBS3079	Fast Mouse GM-CSF ELISA
TBS3080	Fast Mouse G-CSF ELISA
TBS3084	Fast Mouse IFN-γ ELISA
TBS3085	Fast Mouse TGF ELISA
TBS3086	Fast Mouse MCPT-1 ELISA
TBS3090	Fast Mouse IL-17AF ELISA
TBS3091	Fast Mouse IL-19 ELISA
TBS3092	Fast Mouse C3D1 ELISA
TBS3093	Fast Mouse C3D2 ELISA
TBS3094	Fast Mouse C3D3 ELISA
TBS3095	Fast Mouse C3D7 ELISA
TBS3096	Fast Mouse C3D8B ELISA
TBS3097	Fast Mouse IL-33 ELISA
TBS3098	Fast Mouse Insulin ELISA

Fig.3 Mouse C3d Standard Curve



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