Fast Human Complement C4 ELISA

For the quantitation of human complement C4 concentrations in cell culture supernatants, serum, and plasma.

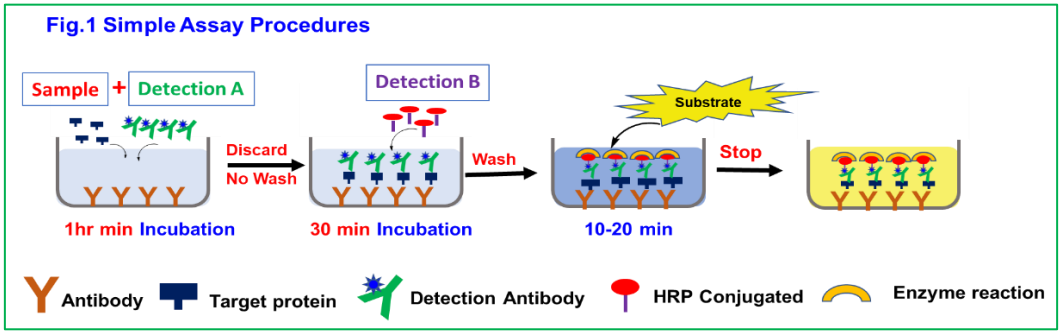
INTRODUCTION

Complement component 4 (C4) plays a key role in the activation of the classical complement pathway. C4 is synthesized as a single-chain precursor molecule (200 kDa) but processed to the three-chain structure with alpha (93 kDa), beta (78 kDa), and gamma (33 kDa) chains prior to secretion. After activation by C1s, C4 is processed to C4a and C4b. C4a anaphylatoxin is a mediator of local inflammation and induces smooth muscle contraction. C4b, the major activation product, is an essential subunit of the C3 and C5 convertases of the classical complement pathway. C4 deficiency is associated with systemic lupus erythematosus. The C4b degradation product, C4d, is a marker for humoral rejection in allografts.

The Fast Human Complement C4 ELISA is a solid phase ELISA designed to measure human complement C4 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 traditional methods. It makes the assay simple, easy, accurate and fast. The measurement can be finished in 1 hour, not need 4-5 hours (Fig. 1). The detection range is from 1 to 300 ng/mL. The levels of human complement C4 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 human complement C4 protein.

PRINCIPLE OF THE ASSAY

This assay employs our novel proprietary sandwich enzyme immunoassay techniques (See Fig. 1). A monoclonal antibody specific for human complement C4 was pre-coated onto a microplate. Standards or samples and Detection Antibody are pipetted into the wells, and concurrently incubated for 1 hour. Then, 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 complement C4 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
Human Complement C4 Microplate	TBS3201A	96 well microplate (12 strips of 8 wells) coated with a Capture Antibody specific for human complement C4.	The unused wells can be stored the sealed foil pouch containing the desiccant pack for up to 1 month at 2-8 °C.
Human Complement C4 Standard	TBS3201B	100 µL of Recombinant human complement C4 protein (3 µg/mL).	Aliquot and store at -20 °C for up to 1 month in a manual defrost freezer. Avoid repeated freeze-thaw cycles.
Detection A	TBS3201C	2.1 ml of human Complement C4 antibody-Biotin.	May be stored for up to 3 months at 2-8 °C.*
Detection B	TBS3201D	100 µL ml of Streptavidin-HRP (100x)	
Assay Diluent	TBS3201E	20 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.

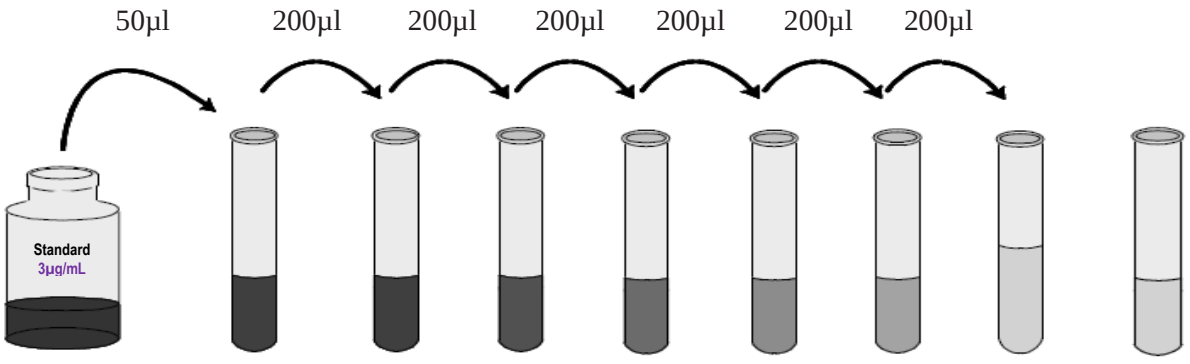
Wash Buffer: Add 10 mL of Wash Buffer Concentrate (10x) to 90 mL of deionized distilled water to prepare 100 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.*).

Detection B: Add 100 µL of Detection B stock (100x) to 10mL of Assay Diluent buffer.

Human Complement C4 Standard Preparation:

1. Label test tubes as #1 through #8. Pipet 450 µL of Assay Diluent into tube#1, and 300 µL into tube 2 through 8 **as diagram below (Fig. 2).**
2. Add 50 µL of standard stock of 3µg/mL into the tube #1, and mix. Then, make 2.5x serial dilutions from tube#2 through #7 with sequential transfer of 200 µL from previous concentration to the next concentration. Mix each tube thoroughly before the next transfer. The standard concentration in tube 1 through 7 will be 300; 120; 48; 19.2; 7.68; 3.07 and 1.23 ng/mL. Tube# 8 is Standard 0.

Fig. 2 Diagram for Human Complement C4 Standard Preparation



Standard Label	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	450	300	300	300	300	300	300	300
Addition	Stock	Std1	Std2	Std3	Std4	Std5	Std6	
Addition Vol. (µL)	50	200	200	250	200	200	200	0
Final Conc.(ng/ml)	300	120	48	19.2	7.68	3.07	1.23	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, sample, or control per 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 1 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 30min.**
5. Aspirate each well, and wash for 3 times 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 human Complement C4concentrations 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(R2=0.9999) 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 human complement C4is typically less than 0.5 ng/ml.
The Intra-assay CV is 5.13% the Inter-assay CV is 7.82%.

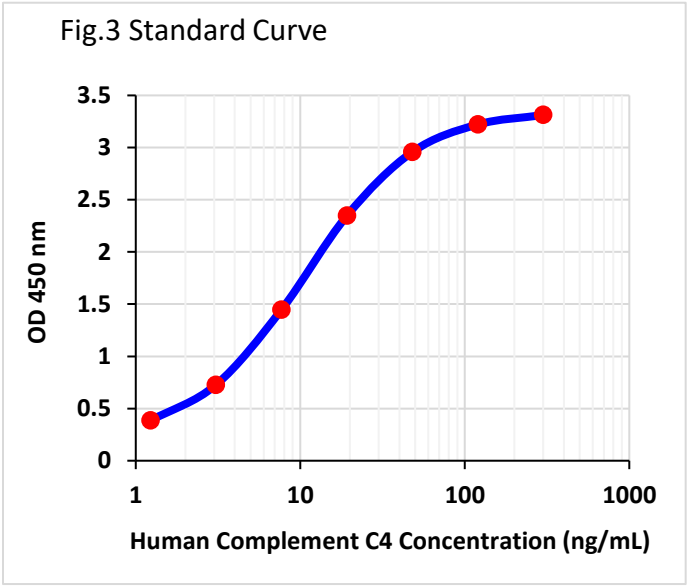
SPECIFICITY

This assay recognizes natural and recombinant human complement C4.
No cross-reactivity with others.

RELATIVE PRODUCTS

Human IL-1β ELISA (TBS3219)
Human IL-2 ELISA (TBS3220)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IL-22 ELISA (TBS3229)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Human TNF-α ELISA (TBS3235)

Fig.3 Standard Curve



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