C4 (Human) ELISA Kit

Catalog Number KA1021

96 assays

Version: 02

Intended for research use only

Table of Contents

Introduction

Background

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 disulphide-linked structure with alpha (93 kDa), beta (78 kDa), and gamma (33 kDa) chains prior to secretion (1, 2). After activation by C1s, C4 is processed to C4a and C4b. C4a anaphylatoxin is a mediator of local inflammation and induces smooth muscle contraction (3). 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 (5). The C4b degradation product C4d is a marker for humoral rejection in allografts (6).

Principle of the Assay.

The C4 (Human) ELISA Kit is designed for detection of human complement C4 in urine, milk, saliva and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human complement C4 in less than 4 hours. A polyclonal antibody specific for human complement C4 has been pre-coated onto a 96-well microplate with removable strips.
Complement C4 in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for Complement C4, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Human Complement C4 Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human complement C4.	96 (8x12) wells
Sealing Tapes: Pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Human Complement C4 Standard: Human Complement C4 in a buffered protein base (1.6 µl, lyophilized).	1.6 µg
Biotinylated Human Complement C4 Antibody (100x): A 100-fold concentrated biotinylated polyclonal antibody against Complement C4.	80 µl
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 ml
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 ml x 2
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate.	80 µl
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 ml
Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction.	12 ml

Storage Instruction

- ✓ Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- ✓ Store SP Conjugate and biotinylated antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 30 days at 2-8°C.
- ✓ Store Standard at 2-8°C before reconstituting with diluent and at -20°C after reconstituting with diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µl, 20-200 µl, 200-1000 µl and multiple channel).
- ✓ Deionized or distilled reagent grade water.

Precautions for Use

- ✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- ✓ This kit is for research use only.
- ✓ The kit should not be used beyond the expiration date.
- ✓ The Stop Solution is an acid solution.

Assay Protocol

Reagent Preparation

- ✓ Freshly dilute all reagents and bring all reagents to room temperature before use.
- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- ✓ Standard Curve: Reconstitute the 1.6 µg Human Complement C4 Standard with 5 ml of MIX Diluent to generate a stock solution of 320 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. The standard stock (320 ng/ml) should be further diluted 1:16 with MIX Diluent to generate a standard solution of 20 ng/ml. Prepare duplicate or triplicate standard points by serially diluting the standard solution (20 ng/ml) 1:4 with MIC Diluent to produce 5, 1.25, 0.313, and 0.78 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Complement C4] (ng/ml)
P1	Standard (320 ng/ml) + 15 parts MIX Diluent	20.00
P2	1 part P1 + 3 parts MIX Diluent	5.000
P3	1 part P2 + 3 parts MIX Diluent	1.250
P4	1 part P3 + 3 parts MIX Diluent	0.313
P5	1 part P4 + 3 parts MIX Diluent	0.078
P6	MIX Diluent	0.000

- ✓ Biotinylated Human Complement C4 Antibody (100x): Spin down the biotinylated antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Sample Preparation

- ✓ Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:2 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles
- ✓ Saliva: Collect saliva using sample pot. Centrifuge samples at 800 x g for 10 minutes. Dilute samples 1:200 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 3000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- ✓ Milk: Collect milk using sample tube. Centrifuge cell culture media at 800 x g for 10 minutes. Milk dilution is suggested at 1:8000 into MIX Diluent; however, the user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Assay Procedure

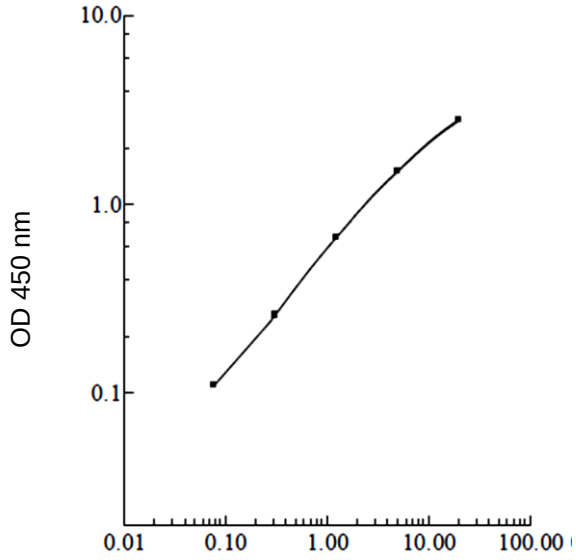
1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µl of Human Complement C4 standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last sample addition.
4. Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent material to completely remove the liquid.
5. Add 50 µl of Biotinylated Human Complement C4 Antibody to each well and incubate for 1 hour.
6. Wash the microplate as described above.
7. Add 50 µl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Human Complement C4 Standard Curve



The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

Performance Characteristics

- ✓ The minimum detectable dose of complement C4 is typically~ 0.075 ng/ml.
- ✓ Intra-assay and inter-assay coefficients of variation were 4.8 % and 7.2 % respectively.

- ✓ Linearity

	Average Percentage of Expected Value
Sample Dilution	Urine
No dilution	91%
1:2	96%
1:4	103%

	Average Percentage of Expected Value
Sample Dilution	Saliva
1:100	96%
1:200	98%
1:400	106%

	Average Percentage of Expected Value
Sample Dilution	Milk
1:4000	97%
1:8000	99%
1:1600	105%

✓ Recovery

Standard Added Value	0.3 – 3 ng/ml
Recovery %	88-109%
Average Recovery %	98%

✓ Cross-Reactivity

Species	% Cross Reactivity
Canine	5%
Bovine	None
Monkey	10%
Mouse	None
Rat	None
Swine	None
Rabbit	None

Resources

References

1. Roos MH et al. (1982) Nature 298(5877):854-856
2. Miura N et al. (1987) J Biol. Chem. 262(15):7298-7305.
3. Belt KT et al. (1984) Cell 36(4):907-914
4. Moon KE et al. (1981) J Biol Chem. 256(16):8685-8692
5. Yang Y et al. (2007) Am J Hum Genet. 80(6):1037-1054
6. Girnita AL et al. (2008) Transplantation 86(2):342-347

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H