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Human Complement C3 ELISA Kit

Catalog # HC3KT

Strip well format. Reagents for up to 96 tests.

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INTENDED USE

This human complement component 3 (C3) total antigen assay is intended for the quantitative determination of total C3 antigen in human plasma, serum, urine, milk, saliva and cell culture samples. **For research use only.**

BACKGROUND

Complement C3, the most abundant serum complement component, is a disulfide-linked 185kDa 1,637 amino acid glycoprotein which supports the classical, alternative, and lectin pathways of complement activation [1]. C3 is proteolytically activated by C3-convertase to the anaphylatoxin C3a and the opsonizing agent C3b [2]. Serum concentrations of C3 are increased during acute and chronic inflammation such as rheumatoid arthritis, and are decreased due to increased consumption or autoimmune disorders such as systemic lupus erythematosus [3].

ASSAY PRINCIPLE

Human C3 will bind to the capture antibody coated on the microtiter plate. After appropriate washing steps, biotin labeled anti-human C3 primary antibody binds to the captured protein. Excess primary antibody is washed away and bound antibody is reacted with peroxidase conjugated streptavidin. Following an additional washing step, TMB substrate is used for color development at 450nm. A standard calibration curve is prepared along with the samples to be measured using dilutions of human C3. Color development is proportional to the concentration of total C3 in the samples.

REAGENTS PROVIDED

- **96-well antibody coated microtiter strip plate** (removable wells 8x12) containing anti-human C3 antibody, blocked and dried.
- **10X Wash buffer:** 1 bottle of 50ml
- **Human C3 standard:** 1 vial lyophilized standard
- **Anti-human C3 primary antibody:** 1 vial lyophilized polyclonal antibody
- **Horseradish peroxidase-conjugated streptavidin:** 1 vial concentrated HRP labeled streptavidin
- **TMB substrate solution:** 1 bottle of 10ml solution

STORAGE AND STABILITY

Store all kit components at 4°C upon arrival. Return any unused microplate strips to the plate pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. Do not freeze-thaw the standard and primary antibody more than once. Store all other unused kit components at 4°C. This kit should not be used beyond the expiration date.

OTHER REAGENTS AND SUPPLIES REQUIRED

- Microtiter plate shaker capable of 300 rpm uniform horizontally circular movement
- Manifold dispenser/aspirator or automated microplate washer
- Microplate reader capable of measuring absorbance at 450 nm
- Pipettes and Pipette tips
- Deionized or distilled water
- Polypropylene tubes for dilution of standard
- Paper towels or laboratory wipes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)

PRECAUTIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR DIAGNOSTIC USE.
- Do not mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
- Always pour peroxidase substrate out of the bottle into a clean test tube. Do not pipette out of the bottle as contamination could result.
- Keep plate covered except when adding reagents, washing, or reading.
- DO NOT pipette reagents by mouth and avoid contact of reagents and specimens with skin.
- DO NOT smoke, drink, or eat in areas where specimens or reagents are being handled.

PREPARATION OF REAGENTS

- **TBS buffer:** 0.1M Tris, 0.15M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer:** Dilute 50ml of 10X wash buffer concentrate with 450ml of deionized water

SAMPLE COLLECTION

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard

Reconstitute standard by adding 1ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. This will result in a 100ng/ml standard solution.

Dilution table for preparation of human C3 standard:

C3 concentration (ng/ml)	Dilutions
10	900µl (BB) + 100µl (from vial)
5	500µl (BB) + 500µl (10ng/ml)
2	600µl (BB) + 400µl (5ng/ml)
1	500µl (BB) + 500µl (2ng/ml)
0.5	500µl (BB) + 500µl (1ng/ml)
0.2	600µl (BB) + 400µl (0.5ng/ml)
0.1	500µl (BB) + 500µl (0.2ng/ml)
0.05	500µl (BB) + 500µl (0.1ng/ml)
0.02	600µl (BB) + 400µl (0.05ng/ml)
0	500µl (BB) Zero point to determine background

NOTE: DILUTIONS FOR THE STANDARD CURVE AND ZERO STANDARD MUST BE MADE AND APPLIED TO THE PLATE IMMEDIATELY.

Standard and Unknown Addition

Remove microtiter plate from bag and add 100µl C3 standards (in duplicate) and unknowns to wells. Carefully record position of standards and unknowns. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

NOTE: The assay measures total human C3 in the 0.02-10 ng/ml range. Samples giving human C3 levels above 10ng/ml should be diluted in blocking buffer before use. A 1:1,000,000 to 1:10,000,000 dilution for normal plasma and serum samples, 1:100 dilution for saliva samples, 1:2 to 1:10 dilution for urine samples, and 1:1,000 to 1:10,000 dilution for milk samples are suggested for best results.

Primary Antibody Addition

Reconstitute primary antibody by adding 10ml of blocking buffer directly to the vial and agitate gently to completely dissolve contents. Add 100µl to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Streptavidin-HRP Addition

Briefly centrifuge vial before opening. Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.4ml of 1:1,000 dilution to 9.6ml of blocking buffer to generate a 1:25,000 dilution. Add 100µl of the 1:25,000 dilution to all wells. Shake plate at 300rpm for 30 minutes. Wash wells three times with 300µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Substrate Incubation

Add 100µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different strengths of blue. Quench reaction by adding 50µl of 1N H_2SO_4 or HCl stop solution to all wells when samples are visually in the same range as the standards. Add stop solution to wells in the same order as substrate upon which color will change from blue to yellow. Mix thoroughly by gently shaking the plate.

Measurement

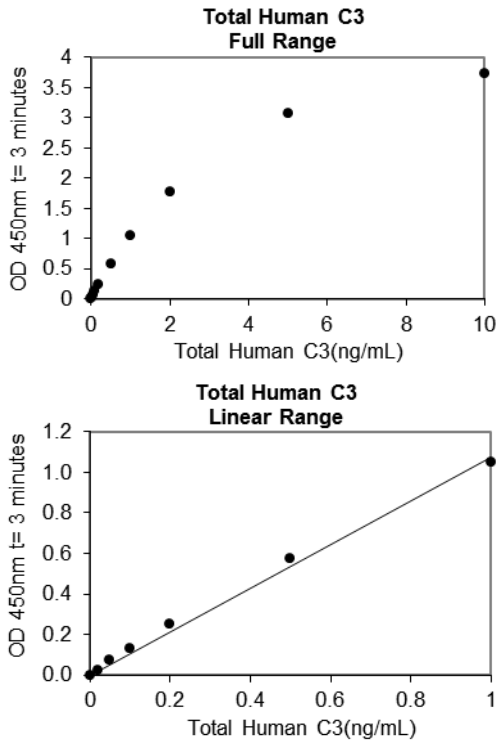
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

Calculation of Results

Plot A_{450} against the amount of C3 in the standards. Fit a straight line through the linear points of the standard curve using a linear fit procedure if unknowns appear on the linear portion of the standard curve. Alternatively, create a standard curve by analyzing the data using a software program capable of generating a four

parameter logistic (4PL) curve fit. The amount of C3 in the unknowns can be determined from this curve. If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

A typical standard curve (EXAMPLE ONLY):



EXPECTED VALUES

C3 in normal human plasma ranges from 0.9-1.9 mg/ml (n=466) with an average concentration of 1.39 mg/ml [4].

PERFORMANCE CHARACTERISTICS

Sensitivity: The minimum detectable dose (MDD) was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates (range OD_{450}: 0.082-0.103) and calculating the corresponding concentration. The MDD was 0.011 ng/mL.

Intra-assay Precision: Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.29	1.44	9.12
Standard Deviation	0.014	0.042	0.302
CV (%)	4.74	2.92	3.31

Inter-assay Precision: Three samples of known concentration were tested in ten independent assays to assess inter-assay precision.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.262	1.40	8.52
Standard Deviation	0.015	0.044	0.443
CV (%)	5.56	3.17	5.19

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in blocking buffer was evaluated.

Sample	1	2	3	4
n	4	4	4	4
Mean (ng/mL)	0.072	0.230	2.30	8.72
Average % Recovery	103	92	92	109
Range	93-113%	80-106%	89-97%	108-110%

Linearity: To assess the linearity of the assay, human plasma samples containing high concentrations of antigen were serially diluted to produce samples with values within the dynamic range of the assay.

Sample	1:2	1:4	1:8	1:16
n	4	4	4	4
Average % of expected	101	111	104	117
Range	95-105%	106-113%	101-106%	113-123%

Specificity: Pooled normal plasma from mouse, rat, pig, sheep, and horse was assayed at the recommended dilution and no significant cross-reactivity was observed.

Sample Values: Samples were evaluated for the presence of the antigen at varying dilutions.

Sample Type	Dilution	Mean (µg/mL)
EDTA Plasma	1:200,000	905
	1:400,000	937
	1:800,000	995
	1:1,000,000	853
	1:2,000,000	889
ACD Plasma	1:1,000,000	977
	1:2,000,000	939
Citrate Plasma	1:1,000,000	957
	1:2,000,000	874
Heparin Plasma	1:1,000,000	683
	1:2,000,000	784
Milk, Centrifuged	1:2,000	17
	1:4,000	18
Milk, Not Centrifuged	1:2,000	15
	1:4,000	15
Urine, Centrifuged	1:2	0.020
	1:4	0.023
	1:8	0.022
Urine, Not Centrifuged	1:2	0.020
	1:4	0.024
	1:8	0.024
Saliva, Centrifuged	1:50	0.222
	1:100	0.297

DISCLAIMER

This information is believed to be correct but does not claim to be all-inclusive and shall be used only as a guide. The supplier of this kit shall not be held liable for any damage resulting from handling of or contact with the above product.

REFERENCES

1. Sahu A and Lambris JD: Immunol Rev. 2001, 180:35-48.
2. Haas PJ and van Strijp J: Immunol Res. 2007, 37:161-175.
3. Unsworth DJ: J Clin Pathol. 2008, 61:1013-1017.
4. Butts WC, *et al.*: Clin Chem. 1977, 23:511-514.

Example of ELISA Plate Layout
96 Well Plate: 20 Standard wells, 76 Sample wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml		
B	0	0.02 ng/ml	0.05 ng/ml	0.1 ng/ml	0.2 ng/ml	0.5 ng/ml	1 ng/ml	2 ng/ml	5 ng/ml	10 ng/ml		
C												
D												
E												
F												
G												
H												