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Human Complement C3 Antigen ELISA Assay

Strip well format. Reagents for up to 96 tests

Catalog No.	**CS409A**	**Quantity:**	**1 X 96 tests**
	CS409B		**5 x 96 tests**

Intended Use: This human complement C3 antigen assay is intended for the quantitative determination of total complement C3 antigen in human plasma, serum, urine, milk, saliva and cell culture samples.

Background: Complement Component 3 (C3), the most abundant serum complement component, is a disulfide-linked 185 kDa 1,637 amino acid glycoprotein which supports the classical, alternative and lectin pathways of complement activation. C3 is proteolytically activated by C3-convertase to the anaphylatoxin C3a and the opsonizing agent C3b. 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.

Assay Principle: Human complement 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 microtiter strip plate:**
  8X12 removable well strips containing anti-human complement 3 antibody on the surface. Strips are blocked and dried.
- **10X Wash Buffer:**
  1 bottle of 50ml; bring to 1x using DI water
- **Human complement C3 standard:**
  1 vial of lyophilized standard
- **Anti-human C3 primary antibody:**
  1 vial of lyophilized antibody
- **Peroxidase conjugated streptavidin:**
  1 vial of concentrated secondary reagent
- **TMB substrate solution:**
  1 bottle of 10ml solution

Storage and Stability: All kit components must be stored at 4°C. Store unopened plate and any unused microtiter strips in the pouch with desiccant. Reconstituted standards and primary may be stored at -80°C for later use. **DO NOT** freeze/thaw the standards and primary antibody more than once. All other unused kit components must be stored at 4°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- 1-channel pipettes covering 20-200 µl, 500-5000 µl and 200-1000µl
- 12-channel pipette for 30-300µl
- Paper towels or kimwipes
- 1.5ml micro centrifuge tubes
- 1N H_2SO_4
- DI water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- TBS buffer
- Blocking buffer
- Microtiter plate spectrophotometer operable at 450nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300rpm

Warnings:

Warning – Avoid skin and eye contact when using TMB substrate solution since it may be irritating to eyes, skin, and respiratory system. Wear safety goggles and gloves.

Precautions:

- **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.
- **DO NOT** pipette reagents by mouth.
- Always pour substrate out of the bottle into a clean test tube. **DO NOT** pipette out of the bottle as you could contaminate the substrate.
- Keep plate covered except when adding reagents, washing, or reading.
- **DO NOT** smoke, drink, or eat in areas where specimens or reagents are being handled.

Preparation of Reagents:

- **TBS buffer:** 0.1 M Tris, 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA in TBS

Sample Preparation:

The assay measures total human complement C3 in the 0.02-10 ng/mL range. Samples giving human C3 levels above 10 ng/mL should be diluted in blocking buffer before use. For best results, dilute plasma and serum samples 1:100,000 to 1:1,000,000, saliva samples to 1:100, urine samples 1:2 to 1:10, and milk samples 1:1,000 to 1:10,000.

Assay Procedure:

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

Preparation of Standard: Reconstitute 100 ng standard vial with 1.0 ml of blocking buffer to give a 100 ng/ml stock solution.

Dilution table for preparation of human C3 standard curve

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C3 concentration (ng/mL)	Dilutions
10	900µL (BB) + 100µL (100ng/mL)
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. Add 100 µl standards in duplicate and unknowns wells. Carefully record position of standards and unknowns. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

Primary Antibody Addition:
Add 10 ml of 1X diluent directly to the primary antibody vial and agitate gently to completely dissolve contents. Add 100 µl to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe.

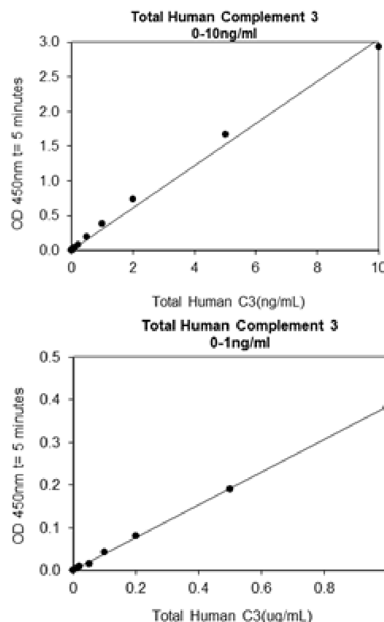
Secondary Reagent Addition:
Dilute 2.5 µl of HRP conjugated streptavidin into 2.5 ml blocking buffer to generate a 1:1,000 dilution. Add 0.2 ml of 1:1,000 dilution to 9.8 ml of blocking buffer to generate a 1:50,000 dilution. Add 100 µl of the 1:50,000 dilution to all wells. Shake plate at 300 rpm for 30 minutes. Wash wells three times with 300 µl wash buffer. Remove excess wash by gently tapping plate on paper towel or kimwipe

Substrate Incubation:
Add 100 µl 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 50 µl of 1N H_2SO_4 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 and read final absorbance values at 450nm. For best results, read plate immediately

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

Assay Calibration:
Plot A_{450} against the amount of C3 in the standards. Fit a straight line through the linear points of the standard curve points 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 total human complement 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, DO NOT USE)



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.

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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 and calculating the corresponding concentration. The MDD was 0.0117 ng/mL.

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

	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: These studies are currently in progress. Please contact us for more information.

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: These studies are currently in progress. Please contact us for more information.

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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
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 or from contact with the above product.

Example of Plate Layout

96 Well Plate
Standards: 20 wells
Samples: 76 wells

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	3.125ng/ml	6.25ng/ml	12.5ng/ml	25ng/ml	50ng/ml	100ng/ml	200ng/ml	400ng/ml	800ng/ml		
B	0	3.125ng/ml	6.25ng/ml	12.5ng/ml	25ng/ml	50ng/ml	100ng/ml	200ng/ml	400ng/ml	800ng/ml		
C												
D												
E												
F												
G												
H												

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