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Human Total Complement C3 ELISA Kit

Catalog No: CS409A
CS409B

Size: 1 x 96 tests
5 x 96 tests

Sensitivity:	0.0148 ng/ml
Specificity:	Human Complement C3 total antigen
Range:	0.02 - 10 ng/ml
Sample Type:	Human serum, plasma, urine, milk, saliva and cell culture samples.
Cross-Reactivity:	Pooled normal plasma from mouse, rat, pig, sheep, and horse were assayed and no significant cross-reactivity was observed.

Background: Complement Component 3 (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. 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: C3 in normal human plasma ranges from 0.9-1.9 mg/ml with an average concentration of 1.39 mg/ml. The assay measures **total human C3** in the 0.02-10 ng/ml range. 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 streptavidin conjugated to HRP. Following an additional washing step, TMB substrate is used for color development at 450nm. Color development is proportional to the concentration of C3 in the samples. A standard calibration curve is prepared using dilutions of purified C3 and is measured along with the test samples.

Reagents Provided:

Description	Quantity
CS409A – P. 96-well microtiter strip plate coated with anti-Human C3 antibody, blocked and dried on well surfaces	1 plate: 96 wells (12 strips x 8 wells)
CS409A - A. Wash Buffer Concentrate (10x)	1 bottle, 50 mL
CS409A - B. Human C3 Standard, lyophilized	1 vial
CS409A - C. Anti-Human C3 primary antibody, lyophilized polyclonal antibody	1 vial
CS409A - D. Horseradish peroxidase-conjugated Streptavidin, concentrated	1 vial
CS409A - E. TMB substrate solution	1 bottle, 10 ml

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Storage and Stability:

All kit components must be stored at 2-8°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 2-8°C. Kit should be used no later than the expiration date.

Reagents and Equipment Required:

- Pipettes covering 0-10 µl and 200-1000 µl, and tips
- 12-channel pipette covering 30-300µl
- Paper towels or laboratory wipes
- Polypropylene conical 50 ml tubes, 1.5 ml flip-cap tubes
- 1N H_2SO_4 or 1N HCl
- Bovine Serum Albumin Fraction V (BSA)
- Tris(hydroxymethyl)aminomethane (Tris)
- Sodium Chloride (NaCl)
- Deionized or distilled water
- Magnetic stirrer and stir-bars
- Plastic containers with lids
- Microtiter plate spectrophotometer operable at 450 nm
- Microtiter plate shaker with uniform horizontally circular movement up to 300 rpm
- Automatic plate washer or wash bottle

Warnings:

Warning – Avoid skin and eye contact when using TMB One 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:** 0.1 M Tris 0.15 M NaCl, pH 7.4
- **Blocking buffer (BB):** 3% BSA (w/v) in TBS
- **1X Wash buffer concentrate:** Dilute 50 ml of 10X wash buffer with 450 ml deionized water

Specimen Collection:

Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or aliquot and store at $\leq$ - 20°C. Avoid repeated freeze-thaw cycles.

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Assay Procedure: Allow microtiter strips and assay components to warm to room temperature for 30 minutes. Perform assay at room temperature. Vigorously shake plate (300rpm) at each step of the assay.

Preparation of Standard:
Reconstitute Standard by adding **1.0 ml of blocking buffer** directly to the vial and agitate gently to completely dissolve contents. This will result in a **100 ng/ml Standard solution**.

Table 1: Dilution table for preparation of Human C3 Standards:

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

NOTE: Dilutions for the standard curve must be made and applied to the plate immediately.

Standard and Unknown Addition:
Remove microtiter plate from bag. Add 100 µl of C3 standards in duplicate and unknowns to 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 laboratory wipes.

NOTE: The assay measures total Human C3 in the 0.02 - 10 ng/ml range. If the unknown is thought to have C3 levels above 10 ng/ml, dilution should be made in blocking buffer: 1:1,000,000 to 1:10,000,000 dilution for normal serum and plasma 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.

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Primary Antibody Addition:
Reconstitute antibody by adding **10 ml blocking buffer** to vial. 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 laboratory wipe.

Streptavidin-HRP Addition:
Briefly centrifuge vial before opening. **Dilute 2.5 µl** of HRP-conjugated streptavidin into 2.5 ml blocking buffer to generate a 1:1,000 dilution. Add **0.1 ml of 1:1,000** dilution to **9.9 ml of blocking buffer** to generate a 1:100,000 dilution. Add 100 µl of the 1:100,000 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 laboratory wipe.

Substrate Incubation:
Add 100 µl TMB substrate to all wells and shake plate for 2-10 minutes. Substrate will change from colorless to different intensities of blue. Quench reaction by adding 50 µl 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 and 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 Human 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.

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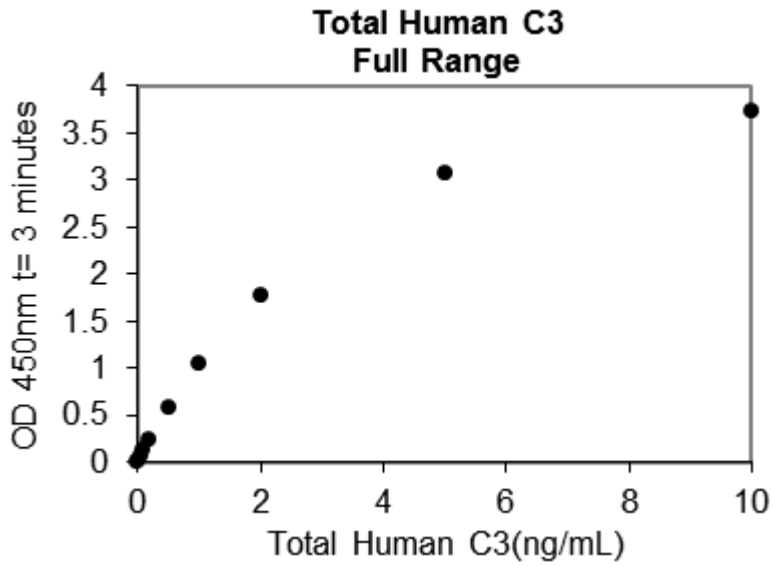
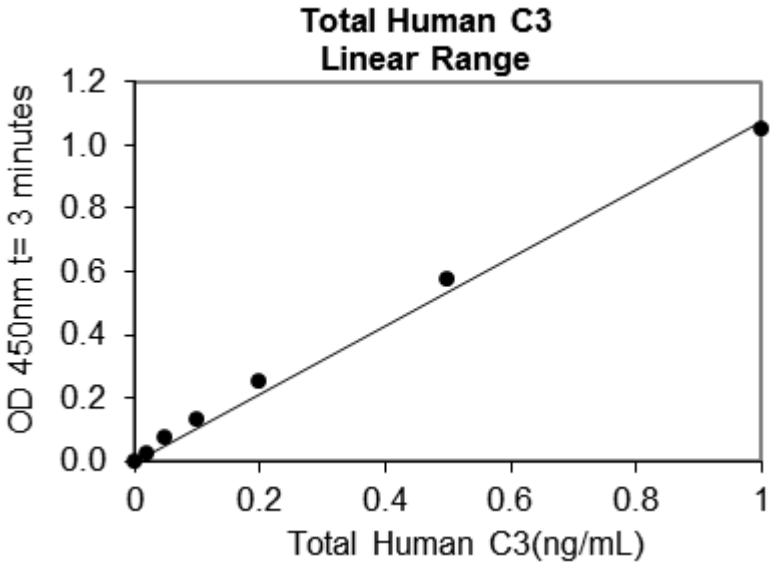
A typical standard curve.
(EXAMPLE ONLY, DO NOT USE)





Expected Values: The average concentration of C3 in normal Human plasma is 0.9 – 1.9 mg/ml (n=466) with an average concentration of 1.39 mg/ml.

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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}: 0.106-0.122) and calculating the corresponding concentration. The MDD was 0.0148 ng/ml.

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

Intra-assay Precision: 3 samples of known concentration were tested 20 times on 1 plate to assess intra-assay precision.

Sample	1	2	3
n	20	20	20
Mean (ng/ml)	0.29	1.44	91.2
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 10 independent assays.

Sample	1	2	3
n	10	10	10
Mean (ng/ml)	0.262	1.40	9.12
Standard Deviation	0.015	0.042	0.443
CV (%)	5.56	3.17	5.19

Recovery: The recovery of antigen spiked to levels throughout the range of the assay in depleted plasma 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 (%)	113	80-106	89-97	108-110

Disclaimer: This information is believed to be correct but does not claim to be all-inclusive and should 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.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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