Human Complement C3 ELISA Kit

Catalog No.: IRAPKT041 **Lot No.:** 1112

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.

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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 block 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 -70°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

Sample Collection

The assay measures total human complement 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. 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.

Reagent Preparation

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

Assay Procedure

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

Preparation of Standard:

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

Dilution table for preparation of human C3 standard curve:

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

Assay Procedure Continued

Standard and Unknown Addition:

Remove microtiter plate from bag. Add 100µl standards in duplicate and unknowns 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.

Primary Antibody Addition:

Add 10ml of blocking buffer directly to the primary antibody 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

Secondary Reagent Addition:

Dilute 2.5µl of HRP conjugated streptavidin into 2.5ml blocking buffer to generate a 1:1,000 dilution. Add 0.2ml of 1:1,000 dilution to 9.8ml of blocking buffer to generate a 1:50,000 dilution. Add 100µl of the 1:50,000 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 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

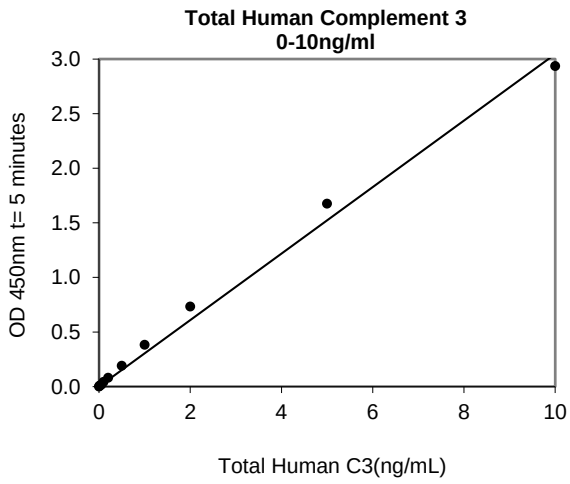
Measurement:

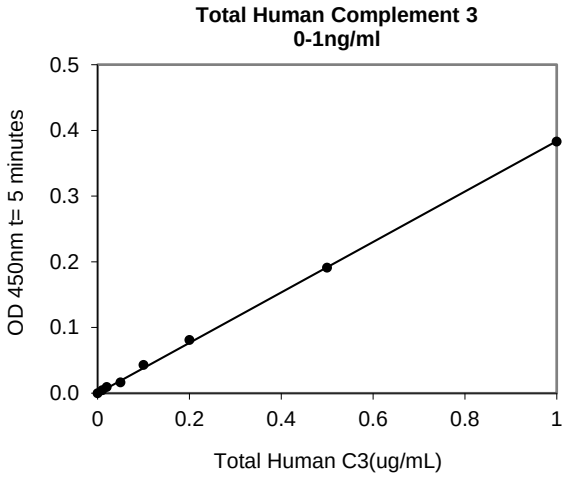
Set the absorbance at 450nm in a microtiter plate spectrophotometer. Measure the absorbance in all wells at 450nm. Subtract zero point from all standards and unknowns to determine corrected absorbance (A_{450}).

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].

Standard Curve Examples





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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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Performance Characteristics

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