C1q Ab ELISA Kit

Catalog Number KA1274

96 assays

Version: 05

Intended for research use only

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Introduction

Intended Use

C1q Ab ELISA Kit is a test system for the quantitative measurement of IgG class autoantibodies against C1q in human serum or plasma. This product is intended for research use only.

Background

The test is used as an aid in the differential diagnosis of systemic autoimmune diseases with renal involvement, e.g. systemic lupus erythematosus, lupus nephritis. Evaluation of a test result should always take into account all clinical and laboratory findings.

Principle of the Assay

Highly purified human C1q is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyzes the substrate forming a blue colored product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

General Information

Materials Supplied

List of component

Component	Package size
Divisible microplate consisting of 12 modules of 8 wells each. Ready to use.	96 wells
Calibrators A-F (0, 6.3, 12.5, 25, 50, 100 U/mL), containing C1q antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.	1.5 mL x 6
Control positive (1) and negative (2), containing C1q antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow, Ready to use. The concentration is specified on the certificate of analysis.	1.5 mL x 2
Sample buffer, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow, concentrate (5x).	20 mL
Enzyme conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 0.05%, light red. Ready to use.	15 mL
TMB substrate, containing 3,3',5,5'-Tetramethylbenzidin, colorless. Ready to use.	15 mL
Stop solution, contains acid. Ready to use.	15 mL
Wash Buffer, containing Tris, detergent, preservative NaN_3 0.09%; 50x concentrated.	20 mL

Storage Instruction

- ✓ Store test kit at 2-8°C in the dark.
- ✓ Do not expose reagents to heat, sun or strong light during storage and usage.
- ✓ Store microplate sealed and desiccated in the clip bag provided.
- ✓ Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- ✓ Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Materials Required but Not Supplied

- ✓ Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- ✓ Data reduction software
- ✓ Multi-Channel Dispenser or repeatable pipet for 100 µL
- ✓ Vortex mixer
- ✓ Pipettes for 10 µL, 100 µL and 1000 µL
- ✓ Laboratory timing device

- ✓ Distilled or deionized water
- ✓ Measuring cylinder for 1000 mL and 100 mL
- ✓ Plastic container for storage of the wash solution
- ✓ This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

Precautions for Use

- Warnings and Precautions
- ✓ All reagents of this kit are intended for professional research use only.
- ✓ Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- ✓ Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- ✓ Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- ✓ Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- ✓ Controls, calibrators, sample buffer and wash buffer contain sodium azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
- ✓ Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
- ✓ During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
- ✓ First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into con-tact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- ✓ Personal precautions, protective equipment and emergency procedures:
- ✓ Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth.
- ✓ Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- ✓ Exposure controls/ personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- ✓ Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- ✓ For disposal of laboratory waste the national or regional legislation has to be observed.
- ✓ Observe the guidelines for performing quality control in laboratories by assaying control sera.

- Procedural Notes
- ✓ Do not use kit components beyond their expiration dates.

- ✓ Do not interchange kit components from different lots and products.
- ✓ All materials must be at room temperature (20-28°C) prior to use.
- ✓ Prepare all reagents and samples. Once started, perform the test without interruption.
- ✓ Double determinations may be done. By this means pipetting errors may become obvious.
- ✓ Perform the assay steps only in the order indicated.
- ✓ Always use fresh sample dilutions.
- ✓ Pipette all reagents and samples into the bottom of the wells.
- ✓ To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- ✓ Wash microwells thoroughly and remove the last droplets of wash buffer.
- ✓ All incubation steps must be accurately timed.
- ✓ Do not re-use microplate wells.

- Limitations of the procedure

  The abnormal and normal reference ranges for antibodies in samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

Assay Protocol

Reagent Preparation

✓ Wash Solution

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 mL prior to use.

✓ Sample Buffer

Sample Buffer: Prior to use dilute the contents (20 mL) of one vial of sample buffer 5x concentrate with distilled or deionized water to a volume of 100 mL.

Sample Preparation

✓ Specimen collection, storage, and handling

1. Collect whole blood specimens using acceptable techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at - 20°C up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.

✓ Preparation of samples

Dilute samples 1:100 before the assay: Put 990 µL of prediluted sample buffer in a polystyrene tube and add 10 µL of sample. Mix well.

Note: Calibrators/ Controls are ready to use and need not be diluted.

Assay Procedure

Prepare enough microplate modules for all calibrators/ controls and samples.

1. Pipette 100 µL of calibrators, controls and prediluted samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28°C).
3. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
4. Dispense 100 µL of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µL of wash solution.
7. Dispense 100 µL of TMB substrate solution into each well.

8. Incubate for 15 minutes at room temperature.
9. Add 100 µL of stop solution to each well of the modules.
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results.
12. The developed colour is stable for at least 30 minutes. Read during this time.

✓ Validation

Test results are valid if the optical densities at 450 nm for calibrators/ controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

Data Analysis

Calculation of Results

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of samples may then be estimated from the calibration curve by interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Performance Characteristics

- ✓ Calibration
  This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

- ✓ Measuring range
  The calculation range of this ELISA assay is 0-100 U/mL.

- ✓ Expected values
  In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 10 U/mL.

- ✓ Interpretation of results
  Negative: $<$ 10 U/mL
  Positive: $\geqq$ 10 U/mL

✓ Linearity

Samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and upper/ lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observe [U/mL]	Expected [U/mL]	O/E [%]
1	1:100	88.4	88.4	100
-	1:200	43.8	44.2	99
-	1:400	22.7	22.1	103
-	1:800	11.5	11.1	104
	1:1600	5.4	5.5	98
2	1:100	65.2	65.2	100
-	1:200	32.1	32.6	98
-	1:400	16.1	16.3	99
-	1:800	7.9	8.2	97
	1:1600	3.7	4.1	91

✓ Limit of detection

Functional sensitivity was determined to be: 0.5 U/mL.

✓ Reproducibility

Intra-Assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Intra-Assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different run. Results for run-to-run precision are shown in the table below.

Intra-Assay			Inter-Assay		
Sample	Mean [U/mL]	CV [%]	Sample	Mean [U/mL]	CV [%]
1	25.2	3.7	1	22.0	4.8
2	58.6	3.0	2	33.2	2.5
3	75.4	2.9	3	53.3	1.9

✓ Interfering Substances

No interference has been observed with haemolytic (up to 1000 mg/dL) or lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

✓ Study results

Study population	n	n Pos	%
Lupus nephritis	34	29	85.3
Systematic Lupus Erythematosus	70	40	57.1
Other disease	91	13	14.3
Normal human serum	74	4	5.4

	Pos	Neg	
Pos	69	17	
Neg	35	148	
	104	165	269

Sensitivity: 66.3%

Specificity: 89.7%

Overall agreement: 80.7%

Resources

References

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Plate Layout

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