anti-C1q Autoantibodies

ELISA

This product is for research use only
It is not intended for use in diagnostic procedures

EK-AC1QA-U 96 tests

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BÜHLMANN LABORATORIES AG
Baselstrasse 55
CH - 4124 Schönenbuch, Switzerland
Tel.: +41 61 487 12 12
Fax: +41 61 487 12 34
info@buhlmannlabs.ch

ENGLISH

INTENDED USE

The BÜHLMANN anti-C1q Autoantibodies ELISA is intended for the quantitative *in vitro* diagnostic determination of anti-C1q autoantibodies in human serum or plasma (1-4). This product is for research use only. It is not intended for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

Calibrators, controls, and sera or plasma containing anti-C1q autoantibodies are incubated with human C1q adsorbed onto microtiter wells. After a washing step, a horseradish peroxidase (HRP) labeled conjugate is added, which binds to the human IgG. After a second washing step, the enzyme substrate (TMB) is added to the wells. Blue color develops in proportion to the amount of anti-C1q autoantibodies bound in the initial step. The reaction is terminated by the addition of stop solution and the color turns from blue to yellow. The absorbance is measured in a microtiter plate reader at a wavelength of 450 nm.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Microtiter Plate Precoated with human C1q	12 x 8 wells	B-AC1QA-MP	Ready to use
Plate Sealer	3 pieces		
Wash Buffer Concentrate (10x) with preservatives	1 vial 100 ml	B-AC1QA-WB	Dilute with 900 ml of deionized water
Incubation Buffer with preservatives	1 vial 100 ml	B-AC1QA-IB	Ready to use
Calibrators A to D Human serum matrix with preservatives	4 vials 1 ml	B-AC1QA-CASET	Ready to use
Controls Low / High Human serum matrix with preservatives	2 vials 1 ml	B-AC1QA-CONSET	Ready to use
Enzyme Label Anti-human IgG-HRP conjugate, with preservatives	1 vial 11 ml	B-AC1QA-ELG	Ready to use
TMB Substrate TMB in Citrate buffer with H_2O_2	1 vial 11 ml	B-TMB	Ready to use
Stop Solution 0.25 M sulfuric acid	1 vial 11 ml	B-STS	Ready to use **Corrosive agent**

Table 1

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
Store at 2-8°C. Do not use past kit expiration date	
Opened / Reconstituted Reagents	
Microtiter Plate	Reseal unused strips immediately to the foil pouch containing the desiccant packs and reseal along the entire edge of zip-seal. Store until expiration date at 2-8°C.
Wash Buffer	Store for up to 3 months at 2-8°C after dilution.
Calibrators	Store at 2-8°C until expiration date.
Controls	
Incubation Buffer	
Enzyme Label	
TMB Substrate	
Stop Solution	Stable at 18-28°C until expiration date marked on the vial.

Table 2

WARNINGS AND PRECAUTIONS

- The Microtiter strips, Calibrators and Controls of this kit contain components of human origin. Each serum donor unit used in the preparation of the kit components was tested by an FDA approved method and found negative for HBV surface antigen antibodies, so as for HCV and HIV1/2 antibodies. Although these methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. Therefore, all samples and kit components should be handled as if capable of transmitting infections. All products containing human source material should be handled in accordance with Good laboratory practice (GLP) using appropriate precautions.
- **Substrate and Stop Solution:** The TMB substrate (B-TMB) contains tetramethylbenzidine (TMB), hydrogen peroxide (H_2O_2) and dimethylformamide. The Stop Solution (B-STS) contains sulfuric acid. Each of those reagents is irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. After contact with eyes or skin wash with plenty of water.

MATERIALS REQUIRED BUT NOT PROVIDED

- 10 µl, 100 µl and 1000 µl precision pipettes with disposable tips.
- Disposable polystyrene or polypropylene tubes for the preparation of sample dilutions.
- 1000 ml cylinder for the dilution of the Wash Buffer.
- Microtiter plate washer or squeeze bottle for Wash Buffer.
- Microtiter plate rotator.
- Blotting paper.
- Microtiter plate reader for measurement of absorbance at 450 nm.

SPECIMEN COLLECTION AND STORAGE

The procedure calls for 50 µl of blood per duplicate determination. Collect blood into plain tubes, avoid hemolysis, mix by inverting sample tube several times and leave to clot for 45 minutes at room temperature (18-28°C) protected from light. Centrifuge at 1800 x g for 15 minutes at room temperature (18-28°C) and collect the serum.
Lipemic, hemolytic and icteric samples should not be used in this assay. Lipemic samples can be avoided by asking to fast for at least 12 hours prior to the sample being taken.
Samples may be stored at 2-8°C for up to 30 days. If samples are stored for a longer period of time they are stable at –20°C for at least 6 months. NOTE: Do not store diluted samples!

PROCEDURAL NOTES

- The enzyme used as the label is inactivated by oxygen and is highly sensitive to sodium azide, thimerosal, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Therefore, use only deionized high quality water.
- It is recommended to assay each control and specimen in duplicate each time a test is performed. Since conditions vary from assay to assay, a new standard curve must be generated each time a new assay is performed. Vertical alignment is recommended.
- If the initial concentration of an unknown sample reads greater than the highest Calibrator (Calibrator A), the sample should be further diluted with Incubation Buffer and assayed again according to the assay procedure. The resulting dilution factor must be accounted for the final calculations.

ASSAY PROCEDURE

Allow all reagents to come to 18-28°C prior to use.

If the Assay is performed at higher Temperatures than 28°C the Results may be wrong. The best Assay Temperature is between 20 and 25°C

1. Dilute samples 1:50 with Incubation Buffer (e.g. 10 µl of serum or plasma + 490 µl of Incubation Buffer) and mix well. Allow diluted samples to stand for 15 minutes at 18-28°C prior to pipetting in step 4c.
2. Prepare a plate with sufficient strips to test the required number of Calibrators, Controls and samples. Remove excess strips from the holder and reseal them in the foil pouch together with the desiccant packs **without delay** and store refrigerated.
3. Wash the coated wells twice using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

4a. Pipet 100 µl of Incubation Buffer in duplicate into A1+A2.
Pipet 100 µl of Calibrator A in duplicate into B1+B2.
Pipet 100 µl of Calibrator B in duplicate into C1+C2.
Pipet 100 µl of Calibrator C in duplicate into D1+D2.
Pipet 100 µl of Calibrator D in duplicate into E1+E2.

4b. Pipet 100 µl of the Control Low in duplicate into F1+F2.
Pipet 100 µl of the Control High in duplicate into G1+G2.

4c. Pipet 100 µl of each diluted sample in duplicate into the subsequent wells.

5. Cover the plate with a plate sealer, place the plate on a plate rotator set at 400-600 rpm and incubate for 1 hour ± 5 minutes at 18-28°C.
6. Remove and discard the plate sealer. Empty the wells and wash three times using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
7. Pipet 100 µl of Enzyme Label into all wells.
8. Cover the plate with a plate sealer, place the plate on a plate rotator set at 400-600 rpm and incubate for 30 ± 5 minutes at 18-28°C.
9. Remove and discard the plate sealer. Empty the wells and wash three times using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
10. Pipet 100 µl of the TMB Substrate into all wells.
11. Cover the plate with a plate sealer, place the plate on a plate rotator set at 400-600 rpm, protect the plate from direct light and incubate for 30 ± 5 minutes at 18-28°C.
12. Pipet 100 µl of Stop Solution to all wells. Remove air bubbles with a pipette tip. Proceed to step 13. Within 30 minutes.
13. Read the absorbance at 450 nm in a microtiter plate reader.

LIMITATIONS

- The reagents supplied with this kit are optimized to measure anti-C1q autoantibodies in human serum or plasma.
- Serum or plasma titer values depend on the assay method and, in particular, on the specificity and the cut-off values established with an assay method. Titer values obtained with different assay methods cannot be compared directly.

RESULTS AND CALCULATION

Standard Curve: Record the absorbance at 450 nm for each Calibrator and blank well.

- Average the duplicate values, subtract the average of the blank wells and record averages (= corrected average absorbance).
- Plot the absorbance (vertical axis) versus the autoantibody titer of the Calibrator (horizontal axis) using lin/log graph paper.

Draw the best fitting curve or calculate the standard curve using a four parameter algorithm.

Samples and Controls: Record the absorbance at 450 nm for each sample and Control well.

- Average the duplicate values, subtract the average of the blank wells and record the averages (=corrected average absorbance).
- Locate the corrected absorbance value of the sample on the vertical axis, draw a horizontal line intersecting the standard curve and read the autoantibody titer (units/ml) from the horizontal axis.

See Table 3 and Figure 1 for typical data (results and standard curve). These results and standard curve are provided for demonstration purposes only. A standard curve must be generated for each set of samples to be assayed.

QUALITY CONTROL

A thorough understanding of this package insert is necessary for the successful use of the product. Reliable results will be obtained only by using precise laboratory techniques (current GLP guidelines) and accurately following this package insert.

Since there is no control serum for anti-C1q autoantibodies commercially available, we recommend using a positive and a negative serum pool for internal quality control.

The reproducibility of standard curve parameters and control values should be within established limits of laboratory acceptability. The confidence limits for the Controls are lot-specific and printed on the QC data sheet added to the kit.

If the precision of the assay does not correlate with the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) ELISA reader settings iii) expiration dates of reagents iv) storage and incubation conditions v) TMB Substrate should be colorless vi) purity of water.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision (Within-Run): 5.0%. The intra-assay precision was calculated from the results of 20 pairs of values from each sample in a single run. The values are presented in Table 4 as units/ml of anti-C1q autoanti-bodies.

Inter-Assay Precision (Run-to-Run): 10.8 %. The inter-assay precision was calculated from the results of 5 pairs of values in 20 different runs. The values are presented in Table 5 as units/ml of anti-C1q autoantibodies.

Sensitivity: 1.0 unit/ml. Twenty duplicates of incubation buffer (=blank) were assayed in a single run. Mean and standard deviation were calculated for the absorbance values. The minimum detectable dose was calculated for the absorbance values. The minimal detectable dose of anti-C1q autoantibodies was calculated to be 1.0 unit/ml by adding two standard deviations to the mean absorbance and intersecting this value with the standard curve obtained in the same run.

Dilution Linearity/Parallelism: 105.9%. Three human serum samples showing high titers of anti-C1q autoanti-bodies were diluted with Incubation Buffer and subsequently assayed according to the assay procedure. The values are presented in Table 6 as units/ml of anti-C1q autoantibodies.

Table 3: **Typical Data**

	Conc. (units/ml)	Abs. (OD)	Calc. Conc. (units/ml)	CV Conc. (%)
Blank		0.068		
Blank		0.068		
Blank Avg.		**0.068**		
Cal A	400	2.332	418	
Cal A	400	2.264	383	
Cal A Avg.	**400**	**2.298**	**400**	**2.1**
Cal B	100	1.165	104	
Cal B	100	1.102	96	
Cal B Avg.	**100**	**1.133**	**100**	**3.9**
Cal C	25	0.369	25	
Cal C	25	0.369	25	
Cal C Avg.	**25**	**0.369**	**25**	**0**
Cal D	5	0.077	5.0	
Cal D	5	0.079	5.0	
Cal D Avg.	**5**	**0.078**	**5.0**	**1.8**
Control LOW		0.078	5.0	
Control LOW		0.077	5.0	**0.9**
Control L. Avg.		**0.077**	**5.0**	
Control HIGH		1.486	154	
Control HIGH		1.511	158	
Control H. Avg.		**1.498**	**156**	**1.2**

Figure 1: **Standard Curve**

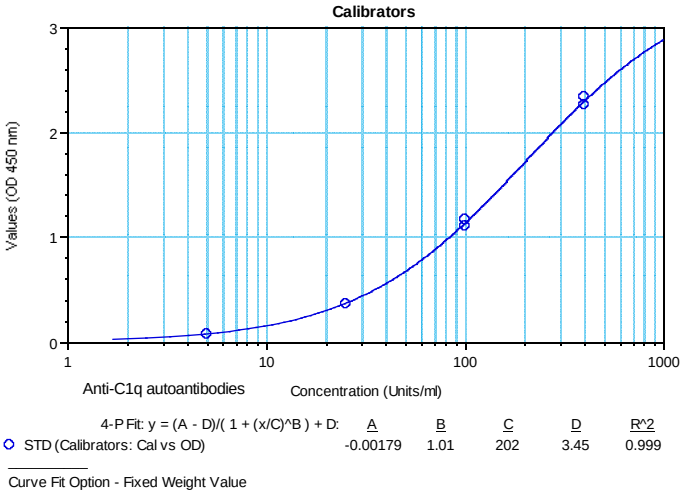

Table 4: **Intra-Assay Precision (Within-Run)**

Sample	Mean [units/ml]	SD [units/ml]	CV [%]
Sample 1	7.8	0.6	7.1
Sample 2	16.7	1.0	6.0
Sample 3	34.7	2.0	5.7
Sample 4	67.0	2.0	3.1
Sample 5	177.0	6.0	3.4
Sample 6	291.0	12.7	4.4
Mean			5.0

Table 5: **Inter-Assay Precision (Run-to-Run)**

Sample	Mean [units/ml]	SD [units/ml]	CV [%]
Sample 7	12.7	1.3	10.3
Sample 8	32.0	4.6	14.3
Sample 9	60.2	6.9	11.5
Sample 10	120.0	8.5	7.1
Sample 11	232.0	25.1	10.8
Mean			10.8

Table 6: **Dilution Linearity/Parallelism**

Sample	Dilution Factor	Observed [units/ml]	Expected [units/ml]	Recovery O/E [%]
Sample 12	1:50	269	--	--
	1:100	145	135	108
	1.200	70.9	67.3	102
	1:400	37.0	33.6	110
	1:800	19.2	16.8	114
	1:1600	10.0	8.4	119
	1:3200	5.1	4.2	121
Sample 13	1:50	276	--	--
	1:100	123	138	91
	1.200	68.6	69.0	99
	1:400	39.9	34.5	116
	1:800	19.6	17.3	114
	1:1600	10.6	8.6	123
	1:3200	5.0	4.3	116
Sample 14	1:50	261	--	--
	1:100	126	130	97
	1.200	65.2	65.3	100
	1:400	32.2	33.6	99
	1:800	15.9	16.3	91
	1:1600	7.0	8.1	86
	1:3200	4.1	4.1	100
Mean				105.9

Table description: cf. “Results and Calculation” (page 3), “Performance characteristics” (page 3)

Table 7: **Cut-Off Values**

	Before elimination	after elemination
Total (n)	220	200
Range	2.0–319	2.0–22.1
Mean	12.1	5.6
Median	4.1	3.8
SD	30.2	4.2
Mean+3SD	102.8	**18.2**

Notes

Notes

APPENDIX II
REFERENCES

1. Siegert CEH *et al*. IgG autoantibodies against C1q are correlated with nephritis, hypocomplementemia, and dsDNA antibodies in systemic lupus erythematosus. J Rheumatol. 1991, 18: 230-234.
2. Siegert CEH et al. Review: Autoantibodies against C1q: view on clinical relevance and pathogenic roles. Clin Exp Immunol 1999, 116: 4-8.
3. Trendelenburg M *et al*. Lack of occurrence of severe lupus nephritis among anti-C1q autoantibody-negative patients. Arthritis Rheum 1999, 41: 187-188.
4. Moroni, G*, et al.* Anti-C1q antibodies may help in diagnosing a renal flare in lupus nephritis Am J Kidney Dis **37**, 490-8. (2001).

APPENDIX III
SHORT PROTOCOL

ANTI-C1Q AUTOANTIBODIES ELISA

Precoated Microtiter Plate

↓ ↺ *wash 2 x*

100 µL Standards, Controls or diluted Samples (1:50)

↓ ↺ *incubate 1 hour ± 5 minutes at 18-28°C on a plate rotator*

↺ *wash 3 x*

add 100 µL Enzyme Label

↓ ↺ *incubate 30 minutes ± 5 minutes at 18-28°C on a plate rotator*

↺ *wash 3 x*

add 100 µL TMB Substrate

↓ ↺ *incubate 30 minutes ± 5 minutes at 18-28°C on a plate rotator*

add 100 µL Stop Solution

→ **Read absorbance at 450 nm (within 30 minutes)**

TIME TO RESULT: 2.0 HOURS

Symbol	Explanation
	Use By
REF	Catalogue number
LOT	Batch code
	Contains sufficient for <n> tests
	Consult Instructions for Use
	Temperature limitation
MP	Microtiterplate
BUF WASH 10X	Wash Bufer Concentrate (10x)
BUF INC	Incubation Buffer

Symbol	Explanation
CAL A	Calibrator A
CAL B	Calibrator B
CAL C	Calibrator C
CAL D	Calibrator D
CONTROL L	Control Low
CONTROL H	Control High
EL	Enzyme Label
SUBS TMB	TMB Substrate
SOLN STOP	Stop Solution

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