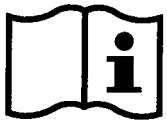
Product information



Userś Manual

LC-1 antibody ELISA

Enzyme Immunoassay for the detection of anti-liver cytosol type 1 autoantibodies (anti-LC-1) in human serum.

REF AE29025

Σ 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

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Version 003
2015-07-21 DWZ
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1 INTENDED USE

This LC-1 antibody ELISA is a solid phase enzyme immunoassay employing human recombinant formiminotransferase-cyclodeaminase (cytosolic liver antigen) for the quantitative and qualitative detection of anti-liver cytosol type 1 autoantibodies (anti-LC-1) in human serum.

For research use only, not for use in diagnostic procedures.

2 INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic progressive liver disease of unknown origin that responds well to immunosuppressive therapy, but has a poor prognosis if untreated. Early and accurate diagnosis is therefore of great importance. AIH is characterized by histological features of periportal hepatitis in the absense of viral markers, by hypergammaglobulinemia and, in the majority of individuals, by the presence of autoantibodies in serum. Anti-nuclear antibodies (ANA), smooth muscle antibodies (SMA), anti-liver kidney microsomal antibodies (LKM) and antibodies against soluble liver antigen (SLA) are marker autoantibodies for AIH. 52% of AIH individuals are positive for ANA and/or SMA, 20% for SLA and 3% for LKM-1. These antibodies are of diagnostic value for AIH but the only autoantibodies highly specific for AIH are SLA. ANA/SMA also occur in 10-15% of individuals with viral hepatitis and other immune-mediated diseases. LKM-1 are also associated with hepatitis C.

3 PRINCIPLE OF THE TEST

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. The IgG antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.

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4 REAGENTS

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Red	Red	Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

5 STORAGE CONDITIONS

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

6 WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[25]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For research use only, not for use in diagnostic procedures.

7 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

8 SAMPLE COLLECTION AND PREPARATION

1. The use of SERUM samples is required for this test. Plasma samples should not be used in this test.
2. Samples should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Samples which cannot be assayed within 24 hours of collection should be frozen at –20 °C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Samples should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing. Samples which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

9 PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel). Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

10 PREPARATION OF REAGENTS AND SAMPLES

All reagents should be brought to room temperature (18 °C - 25 °C) before use.

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 50 µl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

carefully remove liquid by tapping the plate on filter paper. Pipette 300 µl diluted wash buffer in each cavity, wait 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

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11 ASSAY PROCEDURE

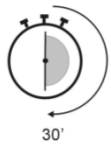
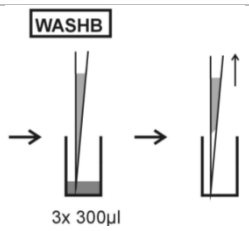
We suggest pipetting calibrators, controls and samples as follows:

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	…	
H	Cal D	NC	…	

Cal A: calibrator A
Cal B: calibrator B
Cal C: calibrator C
Cal D: calibrator D
Cal E: calibrator E
Cal F: calibrator F

	1	2	3	4...
A	NC	P2		
B	NC	P2		
C	CC	P3		
D	CC	P3		
E	PC	…		
F	PC	…		
G	P1	…		
H	P1	…		

PC: positive control
NC: negative control
CC: cut-off calibrator
P1: sample 1
P2: sample 2
P3: sample 3

Step	*Description*
1.	Ensure preparations from step 10 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with the intended quantitative interpretation of the results:
	Calibrators, controls, and samples
3.	 Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either: a. Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* or b. Cut-off Calibrator (CC) for *QUALITATIVE* interp. and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • Diluted sample serum (P1, P2...)
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	 Wash 3x in each case with 300 µl washing buffer (diluted 1:50).

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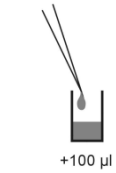
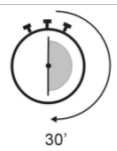
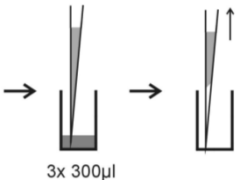
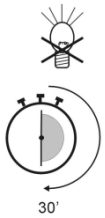
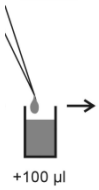
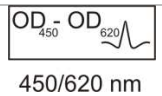
	CONJUGATE	
6.	CONJ 	Pipette 100 µl conjugate into each well.
7.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.	WASHB 	Wash 3x in each case with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.	SUB 	Pipette 100 µl TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	STOP	
11.	STOP 	Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.	OD_{450} - OD_{620}  450/620 nm	Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

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12 INTERPRETATION

For **quantitative interpretation** establish the standard curve by plotting the **optical density** (**OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Evaluation example

This example must not be used to interpret sample results!

Calibrators	OD 450/620 nm	CV % (variance)
0 U/ml	0.046	2.4
3 U/ml	0.171	2.6
10 U/ml	0.372	1.0
30 U/ml	0.698	3.8
100 U/ml	1.456	0.4
300 U/ml	2.396	2.0

Example calculation

Sample	Replication (OD)	Mean (OD)	Result (U/ml)
P 01	1.254/1.208	1.231	74.4
P 02	0.658/0.644	0.651	25.8

Samples that are above the highest calibrator value should be reported as > max. They should be diluted accordingly and be re-evaluated, taking the dilution factor into account. Samples lower than the measurement range should be reported as < min.

For batch-specific data please see the attached QC certificate. Medical laboratories should perform in-house quality controls with their own controls and/or pooled sera according to national legislation.

It is recommended that each laboratory works out its own normal values, based on its own technology, controls, equipment and population.

If the control values do not meet the validation criteria, the test is invalid and must be repeated.

The following technical data should be reviewed: expiry dates of the reagents, storage conditions, pipettes, used equipment, photometer, incubation conditions and washing methods.

If the tested samples reveal unusual values or deviations, or if the validation criteria are not met for inexplicable reasons, please contact the IBL-America.

For **qualitative interpretation** read the optical density of the cut-off calibrator and the samples. Compare all sample ODs with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider samples within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative:		**OD sample**	**<**	**0.8 x OD cut-off**		
Equivocal:	**0.8 x**	**OD cut-off**	**≤**	**OD sample**	**≤**	**1.2 x OD cut-off**
Positive:		**OD sample**	**>**	**1.2 x OD cut-off**		

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13 TECHNICAL DATA

Sample material:	Serum
Sample volume:	10 µl of a 1:101 sample dilution with 1x sample buffer
Total incubation period:	90 minutes at 20-26°C/68-78.8°F.
Measurement range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F in original bottles.
Number of determinations:	96 tests

14 PERFORMANCE DATA

14.1 Analytical Sensitivity

Testing sample buffer 30 times with this LC-1 IgG ELISA gave an analytical sensitivity of 1.0 U/ml.

14.2 Specificity and Sensitivity

The microplate is coated with recombinant human formimidoyltransferase cyclodeaminase. No cross reactivities to other autoantigens have been found.

14.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample no.	Dilution Factor	Measured (U/ml)	Expected (U/ml)	Recovery (%)
1	1 / 100	134.8	135.0	99.8
	1 / 200	66.3	67.5	98.2
	1 / 400	32.8	33.8	97.0
	1 / 800	15.8	16.9	93.5
2	1 / 100	98.5	100.0	98.5
	1 / 200	51.2	50.0	102.4
	1 / 400	25.3	25.0	101.2
	1 / 800	11.5	12.5	92.0

14.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intraassay		
Sample no.	**Mean (U/ml)**	**CV (%)**
1	143.0	3.2
2	83.0	4.1
3	19.0	3.8

Interassay		
Sample no.	**Mean (U/ml)**	**CV (%)**
1	141.0	2.8
2	85.0	4.2
3	23.0	5.1

14.5 Calibration

Due to the lack of international reference calibration this LC-1 Ab ELISA is calibrated in arbitrary units (U/ml).

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15 LITERATURE

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Meyer zum Büschenfelde KH, Lohse AW (1995). Autoimmune Hepatitis. N Engl J Med 333: 1004-1005.

Alvarez F, Berg PA, Bianchi et al. (1999). International Autoimmune Hepatitis Group Report: a review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 31: 929-938.

Manns MP et al. (1991). LKM-1 autoantibodies recognize a short linear sequence in P450 IID6, a cytochrome P-450 monooxygenase. J Clin Invest 88: 1370-1378.

Lapierre P, Hajoui O, Homberg JC, Alvarez F (1999). Formiminotransferase cyclodeaminase is an organ-specific autoantigen recognized by sera of patients with autoimmune hepatitis. Gastroenterology 116: 643-649.

Muratori L, Sztul E, Muratori P, Gao Y, Ripalti A, Ponti C, Lenzi M, Landini MP, Bianchi FB (2001). Distinct epitopes on formiminotransferase cyclodeaminase induce autoimmune liver cytosol antibody type 1. Hepatology 34: 494-501.

Manufactured for :
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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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