DRG® Anti-LC-1 IgG ELISA (EIA-4276)

CE

REVISED 8 MAR. 2011 RM (VERS. 3.1) **RUO IN THE USA**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

Anti-LC-1 is used for determination of IgG antibodies to formiminotransferase-cyclodeaminase in human serum or plasma for the detection of autoimmune hepatitis (AIH).

PRINCIPLE OF THE TEST

Anti-LC-1 is an enzyme immunoassay for determination of IgG antibodies to formiminotransferase-cyclodeaminase.
The antibodies of the standards, controls, and diluted samples react with human recombinant formiminotransferase-cyclodeaminase immobilized on the solid phase of microtiter plates. The use of highly purified human recombinant formiminotransferase-cyclodeaminase guarantees the specific binding of LC-1 antibodies of the specimen under investigation. Following an incubation period of 30 min at room temperature (RT), unbound serum components are removed by a wash step.
The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at RT. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.
HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (HCl) into the wells after 30 min at RT turning the solution from blue to yellow.
The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

SPECIMEN SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at – 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

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Note: ***Samples have to be diluted 1 + 100 (v/v),***
e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A [Ag 96]	Microtiter plate**, 12 breakable strips per 8 wells coated with human recombinant formiminotransferase-cyclodeaminase**	**1** **vacuum sealed with desiccant**
B **[BUF WASH]** **[50X]**	**Concentrated wash buffer, 50X** sufficient for 1000 ml solution	20 ml concentrate capped white
C **[DIL] [5X]**	**Concentrated sample diluent, 5X**	20 ml concentrate capped white
D **[CONJ G]**	**Conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped blue
E **[SOLN TMB]**	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped black
F **[HCL] [1,0M]**	**Stop solution** 1.0 M hydrochloric acid	15 ml ready for use capped white
1 – 6 **[CAL]**	**Standards** (diluted serum) conc.: 0, 3, 10, 30, 100, 300 U/ml	1.5 ml each ready for use
P **[CONTROL]** **[+]**	**Positive control** (diluted serum) conc.: see leaflet enclosed	1.5 ml ready for use capped red
N **[CONTROL]** **[–]**	**Negative control** (diluted serum) conc.: see leaflet enclosed	1.5 ml ready for use capped green

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Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- distilled or de-ionized water

Size and storage

Anti-LC-1 has been designed for 96 determinations.
The expiry date of each component is reported on its respective label that of the complete kit on the box labels.
Upon receipt, all components of the Anti-LC-1 have to be kept at 2 - 8 °C, preferably in the original kit box.
After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.
The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of **wash solution** by diluting the concentrated wash buffer 50 times with de-ionized or distilled water. For example, dilute 1 ml of the concentrate with 49 ml of distilled water per strip.
The wash solution prepared is stable at 2 - 8 °C up to 30 days.
Prepare a sufficient amount of **sample diluent** by diluting the concentrated diluent 5 times with de-ionized or distilled water. For example, dilute 10 ml of the concentrate with 40 ml of distilled water.
The sample diluent prepared is stable at 2 - 8 °C up to 30 days.
Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.
Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C).**
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl standards (1 - 6)
   100 µl positive (P) and negative (N) control
   100 µl diluted samples
   into the respective wells.

3. Incubate **30 min** at room temperature (18-25°C).
4. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) to each well.
6. Incubate **30 min** at room temperature (18-25°C).
7. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **30 min protected from light** at room temperature (18-25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING

We recommend log / lin processing for best results.
The standard curve is established by plotting the mean OD-values of the standards 1 - 6 on the ordinate, y-axis, (lin. scale) versus their respective Anti-LC-1 IgG concentrations on the abscissa, x-axis, (log. scale).
LC-1 antibody concentrations of the unknown samples are directly read off in U/ml against the respective OD values.
Anti-LC-1 may be used also with Computer Assisted Analysis using software able to plot log/lin curves with four-parameter fit.
Using the recommended dilution of 1 + 100 (v/v) for specimen sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Example of Typical Assay Results

Well	OD (a)	OD (b)	OD (mean)	U/ml
Standard 1	0.043	0.045	0.044	0
Standard 2	0.167	0.177	0.172	3
Standard 3	0.363	0.382	0.372	10
Standard 4	0.691	0.705	0.698	30
Standard 5	1.434	1.458	1.446	100
Standard 6	2.383	2.409	2.396	300
Sample 1	0.658	0.644	0.651	26

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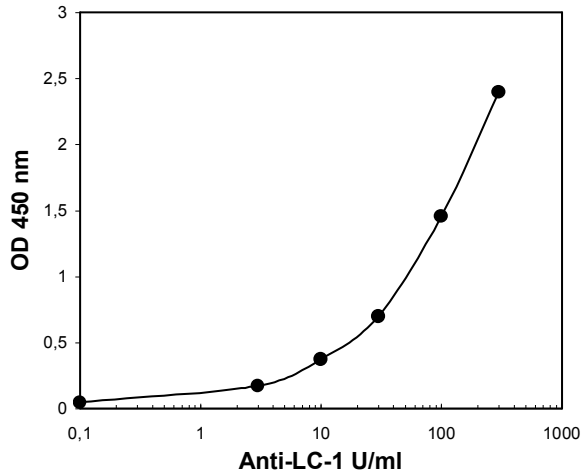
TYPICAL STANDARD CURVE



Specimens with an OD > standard 6, should be diluted with LC-1 antibody negative serum and tested again. Results are multiplied with the dilution factor chosen.
Do not use this example for interpreting results.

Test validity

The test run is valid if:

- the mean OD of the standard 1 is ≤ 0.15
- the mean OD of the standard 6 is ≥ 1.3

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

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INCUBATION SCHEME

Dilute s sample	**10 µl serum + 1.0 ml sample diluent (C)**

1	Bring all ready for use reagents to room temperature (18-25°C) before use.			
2	Pipette Standards (1 - 6)	100 µl		
	Control (P + N)		100 µl	
	prediluted 1 + 100 sera			100 µl
3	Incubate	30 minutes at room temperature (18-25°C)		
4	Wash	Decant, 5 x 300 µl (made of B)		
5	Pipette conjugate (D)	100 µl	100 µl	100 µl
6	Incubate	30 minutes at at room temperature (18-25°C)		
7	Wash	Decant, 5 x 300 µl (made of B)		
8	Pipette substrate (E)	100 µl	100 µl	100 µl
9	Incubate protected from light	30 minutes at room temperature (18-25°C)		
10	Pipette stop solution (F)	100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm within 30 min.			

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SAFETY PRECAUTIONS

- Follow the working instructions carefully. DRG and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

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