LKM-1 Ab ELISA Kit

Catalog Number KA1280

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

The LKM-1 Ab ELISA Kit is a solid phase enzyme immunoassay employing human recombinant cytochrome p450 IID6 for the quantitative and qualitative detection of antibodies against liver-kidney microsomes (LKM) in human serum.

Background

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 patients, 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 patients 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 patients with viral hepatitis and other immune-mediated diseases. LKM-1 are also associated with hepatitis C. Three types of LKM antibodies can be distinguished according to the target antigens. LKM-1 antibodies are directed against cytochrome p450 IID6, a 50 kDa cytoplasmic protein found in hepatocytes and renal proximal tubular cells. LKM-2 antibodies are associated with ticrynafen (tienilic acid) –induced hepatitis. The target antigen is cytochrome p450 IIC9, a cytochrome p450 isoenzyme that catalyzes the metabolic oxidation of the drug. LKM-3 antibodies are associated with chronic hepatitis D. The target antigen is UDP-1 glucoronosyl transferase. LKM-1 associated AIH predominantly occurs in girls between 2 and 14 years of age, thus determination of LKM-1 is very important in pediatrics.

Principle of the Assay

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Individual's 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 samples.

General Information

Materials Supplied

List of component

Component	Description / Contents	Solution color	Amount
Sample Buffer (5x)	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).	Yellow	20 mL
Wash Buffer (50x)	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)	Green	20 mL
Negative Control (Control 2)	Ready to use. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).	Colorless	1.5 mL
Positive Control (Control 1)	Ready to use. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).	Yellow	1.5 mL
Cut-off Control (Control 3)	Ready to use. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).	Yellow	1.5 mL
Calibrator A-F	Ready to use. Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).	Yellow*	1.5 mL x 6
Conjugate IgG	Ready to use. Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA).	Blue	15 mL
TMB Substrate	Ready to use. Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2).	Colorless	15 mL
Stop Solution	Ready to use. 1 M Hydrochloric Acid.	Colorless	15 mL
Microtiter Plate	Ready to use. With breakaway microwells.	N/A	12 x 8 well strips

*Color increasing with concentration

Storage Instruction

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, at least. 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.

Materials Required but Not Supplied

- ✓ Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm).
- ✓ Glass ware (cylinder 100-1000 mL), test tubes for dilutions.
- ✓ Vortex mixer, precision pipettes (10, 100, 200, 500,1000 µL) or adjustable multipipette (100-1000 µL).
- ✓ Microplate washing device (300 µL repeating or multichannel pipette or automated system), adsorbent paper.
- ✓ Our tests are designs 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.).

Precautions for Use

- ✓ Health hazard data

  The product is for research use only. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

- Recommendations and precautions

  This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

  WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth. All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and samples as if capable of transmitting infectious diseases and according to national requirements. The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

- ✓ General directions for use

  Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results. Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test. Incubation: We recommend test performance at 30°C/86°F for automated systems. Never expose components to higher temperature than 37°C/ 98.6°F. Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

Assay Protocol

Reagent Preparation

✓ Preparations prior to starting

- 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.
- 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: Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µL of diluted wash buffer into each well, wait for 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).

Sample Preparation

✓ Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used during the first 8 h, respectively stored tightly closed at 2-8°C/35-46°F up to 48h, or frozen at -20°C/-4°F for longer periods

✓ Sample Preparation

Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µL sample buffer (1x) + 10 µL serum. Mix well !

Assay Procedure

✓ Pipetting Scheme

We suggest pipetting calibrators, controls and samples as Plate Layout.

✓ Test Steps

1. Ensure preparation of reagents and samples from Reagent Preparation and Sample Preparation above have been carried out prior to pipetting.
2. Use the following steps in accordance with quantitative/qualitative interpretations results desired:

3. Pipette controls and samples into the designated wells as described in Plate Layout, 100 µL of either:
    a. Calibrators (Cal A to Cal F) for quantitative or
    b. Cut-off Control for qualitative interp. And 100 µL of each following: Negative control (NC) and Positive control (PC), and diluted serum samples (S1, S2…).
4. Incubate for 30 minutes at 20-32°C/68-89.6°F.
5. Wash 3x with 300 µL washing buffer (diluted 1:50).
6. Pipette 100 µL conjugate into each well.
7. Incubate for 30 minutes at 20-32°C/68-89.6°F.
8. Wash 3x with 300 µL washing buffer (diluted 1:50).
9. 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.
11. 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. Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

Data Analysis

Calculation of Results

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

✓ Example of a standard curve

Do NOT use this example for interpreting sample's result.

Calibrators IgG	OD 450/620 nm	CV % (Variation)
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 of calculation

Sample	Replicate (OD)	Mean (OD)	Result (U/mL)
S 01	0.533/0.569	0.551	19.8
S 02	1.156/1.196	1.176	68.7

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min. For lot specific data, see enclosed quality control leaflet. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and sample population according to their own established procedures. In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated. The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods. If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the supplier of the test kit.

For qualitative interpretation read the optical density of the cut-off calibrator and the samples. Compare sample's OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera 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	

Performance Characteristics

✓ Technical Data

- Sample material: serum
- Sample volume: 10 µL of sample diluted 1:101 with 1x sample buffer
- Total incubation time: 90 minutes at 20-32°C/68-89.6°F
- Calibration range: 0-300 U/mL
- Analytical sensitivity: 1.0 U/mL
- Storage: at 2-8°C/35-46°F use original vials only.
- Number of determinations: 96 tests

✓ Specificity and sensitivity

The microplate is coated with recombinant human cytochrome p450 IID6.

No crossreactivities to other autoantigens have been found.

Testing sample buffer 30 times on LKM-1 Ab ELISA Kit gave a sensitivity of 1.0 U/mL.

✓ 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	78.9	80.0	98.6
	1 / 200	39.8	40.0	99.5
	1 / 400	18.9	20.0	94.5
	1 / 800	9.6	10.0	96.0
2	1 / 100	34.2	33.0	103.6
	1 / 200	17.2	16.5	104.2
	1 / 400	8.1	8.3	97.6
	1 / 800	4.0	4.2	95.2

✓ 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

Intra-Assay		
Sample No.	Mean (U/mL)	CV (%)
1	210.0	1.6
2	77.5	2.8
3	18.4	3.6

Inter-Assay		
Sample No.	Mean (U/mL)	CV (%)
1	207.0	4.2
2	73.8	2.3
3	17.6	1.5

✓ Calibration

Due the lack of international reference calibration this assay is calibrated in arbitrary units (U/mL).

Resources

References

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5. Homberg JC, Andre C, Abuaf A (1984). A new anti-liver-kindney microsome antiboda (anti-LKM-2) in tienilic acid-induced hepatitis. Clin Exp Immunol 55: 561-570.
6. Philipp T, Durazzo M, Trautwein C, Alex B, Straub P, Lamb JG, Johnson EF, Tukey RH, Manns MP (1994). Recognition of uridine diphosphate glucuronosyl transferases by LKM-3 antibodies in chronic hepatitis D. Lancet 344:578-81.

Plate Layout

		For quantitative interpretation						For qualitative interpretation use					
		1	2	3	4	5	6	1	2	3	4	5	6
A		Calibrator A	Calibrator E	Sample 1				Negative Control	Sample 2				
B		Calibrator A	Calibrator E	Sample 1				Negative Control	Sample 2				
C		Calibrator B	Calibrator F	Sample 2				Cut-off control	Sample 3				
D		Calibrator B	Calibrator F	Sample 2				Cut-off control	Sample 3				
E		Calibrator C	Positive Control	Sample 3				Positive Control					
F		Calibrator C	Positive Control	Sample 3				Positive Control					
G		Calibrator D	Negative Control					Sample 1					
H		Calibrator D	Negative Control					Sample 1					