Human LKM1 antibody ELISA Kit

Enzyme Immunoassay for the quantitative determination of Liver kidney microsomal type 1 (LKM1) antibodies in human serum

Catalog number: ARG80365

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Autoimmune liver disease (eg, autoimmune hepatitis and primary biliary cirrhosis) is characterized by the presence of autoantibodies including smooth muscle antibodies (SMA), antimitochondrial antibodies (AMA), and anti-liver/kidney microsomal antibodies type 1 (anti-LKM-1). Subtypes of autoimmune hepatitis (AIH) are based on autoantibody reactivity patterns.

Anti-LKM-1 antibodies serve as a serologic marker for AIH type 2 and typically occur in the absence of SMA and antinuclear antibodies. These antibodies react with a short linear sequence of the recombinant antigen cytochrome monooxygenase P450 2D6. Patients with AIH type 2 more often tend to be young, female, and have severe disease that responds well to immunosuppressive therapy.

Anti-LKM-1 antibodies may occur in some patients with chronic hepatitis caused by hepatitis C virus infection. Although the epitopes recognized by anti-LKM-1 antibodies in hepatitis C virus infection are different than in patients with AIH type 2, physicians must use caution in interpreting the results of tests for anti-LKM-1 antibodies in such patients.

[Information from mayomedicallaboratories.com]

PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique. The Human recombinant cytochrome p450 2D6 protein has been pre-coated onto a microtiter plate. Standards or 1:101 diluted samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti human antibody is

added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	**Quantity**	**Storage information**
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standards A-F (0, 3, 10, 30, 100, 300 U/ml)	6 x 1.5 ml (Ready-to-use)	4°C.
Control 1 (Positive Control)	1.5 ml (Ready-to-use)	4°C.
Control 2 (Negative Control)	1.5 ml (Ready-to-use)	4°C.
Control 3 (Cut-off Control)	1.5 ml (Ready-to-use)	4°C
HRP-Antibody conjugate	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
5X Sample Buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450 nm (optional reference absorbance at 600-690 nm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or store samples at 2-8 °C up to 48 hours, or aliquot and store ≤ -20 °C for longer periods. Avoid repeated freeze-thaw cycles.

Note: Do not use icteric, lipemic, hemolysed or bacterially contaminated samples.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer. (e.g. 4 ml 50x Wash buffer + 196 ml distilled water)
- **1X Sample Buffer**: Dilute 5X Sample Buffer into distilled water to yield 1X Wash buffer. (e.g. 20 ml Sample Buffer + 80 ml distilled water)
- **Patient sample**: Dilute patient sample 1:101 with 1x Sample buffer before assay, mix well. (e.g. 10 µl of serum + 1000 µl of 1x sample buffer)

  Note: the controls / Standards are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. QUANTITATIVE:

   Add 100 µl of calibrators, controls 1 and 2 (positive and negative controls) and diluted samples into wells. Leave one well for substrate blank.

   QUALITATIVE

   Add 100 µl of controls 1, 2 and 3 (positive, negative and cut-off controls) and diluted samples into wells. Leave one well for substrate blank.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100 µl HRP-Antibody conjugate into each well. Incubate for 30 minutes at RT.
6. Wash as according to step 4.
7. Add 100 µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature in the dark.
8. Add 100 µl of Stop Solution to each well. Incubate for 5 minutes (minimum)

at RT. The color of the solution should change from blue to yellow.

9. Read the OD with a microplate reader at 450nm immediately (optional reference absorbance at 600-690 nm).

CALCULATION OF RESULTS

For quantitative 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: < 12 U/ml

Equivocal Range: 12-18 U/ml

Positive Results: > 18 U/ml

- Samples above the highest calibrator range should be reported as >Max, or the samples should be diluted as appropriate and re-assayed
- Samples below calibrator range should be reported as < Min

For qualitative interpretation

- For qualitative interpretation read the optical density of the cut-off calibrator and the patient samples. Compare patient´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.

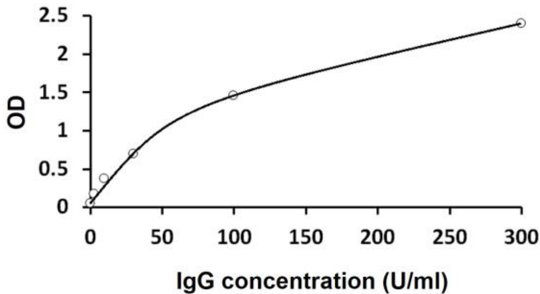
Negative: $OD_{patient} < 0.8 \times OD_{cut\text{-}off}$

Equivocal: $0.8 \times OD_{cut\text{-}off} \leq OD_{patient} \leq 1.2 \times OD_{cut\text{-}off}$

Positive: $OD_{patient} > 1.2 \times OD_{cut\text{-}off}$

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Limit of detection

Functional sensitivity was determined to be 1 U/ml

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.7% and inter-assay precision was 2.7%.

Linearity

94.5 – 104.2%

Specificity and sensitivity

The microplate is coated with recombinant human cytochrome p450 IID6. No cross-reactivities to other autoantigens have been found. Anti-LKM-1 antibodies show a diagnostic specificity of >99% for autoimmune hepatitis type 2. The diagnostic sensitivity of anti-LKM-1 antibodies for autoimmune hepatitis type 2 is 84%.