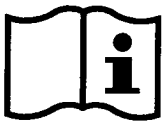
Product information

Information about other products is available at: www.demeditec.com



Useŕs Manual

Anti-LKM-1 ELISA

Enzyme immunoassay for the quantitative and qualitative detection of antibodies against liver-kidney microsomes (LKM) in human serum

CE

IVD

REF **DE7703**

Σ **96 wells**

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1 INTENDED USE

Anti-LKM-1 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. The assay is a tool for the diagnosis of autoimmune hepatitis (AIH).

2 CLINICAL APPLICATION AND PRINCIPLE OF THE ASSAY

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.

2.1 Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient´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 patient sample.

3 KIT CONTENTS

TO BE RECONSTITUTED				
Item	**Quantity**	**Cap color**	**Solution color**	**Description / Contents**
SAM DIL 5x Sample Buffer (5x)	1 x 20 ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
WASH SOLN 50x Wash Buffer (50x)	1 x 20 ml	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**
CONTROL 2 Negative Control	1 x 1.5 ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
CONTROL 1 Positive Control	1 x 1.5 ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
CONTROL 3 Cut-off Control	1 x 1.5 ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
CAL A - F Calibrators	6 x 1.5 ml	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)
ENZ CONJ Conjugate IgG	1 x 15 ml	Blue	Blue	Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
SUB TMB TMB Substrate	1 x 15 ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
STOP SOLN Stop Solution	1 x 15 ml	White	Colorless	1M Hydrochloric Acid
SORB MT Microtiter Plate	12 x 8 well strips	n/a	n/a	With breakaway microwells. Refer to paragraph 1 for coating.
**Color increasing with concentration*				

3.1 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.).

4 STORAGE AND SHELF LIFE

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.

5 PRECAUTIONS OF USE

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended 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 patient 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.

5.2 General directions for use

In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the supplier of the test kit. 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. A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

6 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.

7 ASSAY PROCEDURE

7.1 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.
Samples: Dilute serum samples 1:101 with sample buffer (1x) e.g. 1000 µl sample buffer (1x) + 10 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: 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).

7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For *QUANTITATIVE* interpretation

	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	...	

For *QUALITATIVE* interpretation

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

CalA: calibrator A
CalB: calibrator B
CalC: calibrator C
CalD: calibrator D
CalE: calibrator E
CalF: calibrator F
PC: positive control
NC: negative control
CC: cut-off calibrator
P1: patient 1
P2: patient 2
P3: patient 3

7.3 Test Steps

Step	Description
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting
2.	Use the following steps in accordance with quantitative/qualitative interpretation results desired:
	CONTROLS & 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 patients diluted serum (P1, P2...)
4.	Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	Wash 3x with 300 µl washing buffer (diluted 1:50).
	CONJUGATE
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).
	SUBSTRATE
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.
	STOP
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 (recommended 450/620 nm) within 30 minutes.

8 QUANTITATIVE AND QUALITATIVE 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

Example of a standard curve
Do NOT use this example for interpreting patient'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

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P01	0.533/0.569	0.551	19.8
P02	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. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient 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 manufacturer or the supplier of the test kit. 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 x OD cut-off**	
Equivocal:	**0.8 x**	**OD cut-off**	**≤**	**OD patient**	**≤ 1.2 x OD cut-off**
Positive:		**OD patient**	**>**	**1.2 x OD cut-off**	

9 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

10 PERFORMANCE DATA

10.1 Analytical sensitivity

Testing sample buffer 30 times on Anti-LKM-1 gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity

The microplate is coated with recombinant human cytochrome p450 IID6. No crossreactivities 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%.

10.3 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	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

10.4 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.

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

10.5 Calibration

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

11 LITERATURE

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4. 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.
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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

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	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.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	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	Distributtore