AESKUBLOT Liver Pro

Ref 4004

Instruction Manual

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1 Intended Use

AESKUBLOT Liver Pro is a membrane based enzyme immunoassay for qualitative detection of IgG antibodies against AMA M2, Sp100, LKM1, gp210, LC1 and SLA in human serum or plasma. Antigens are located as parallel lines at exactly defined positions on a nitrocellulose membrane.

The assay is a tool in differential diagnosis of autoimmune liver diseases.

2 Clinical Application and Principle of the Test

The most important autoimmune liver diseases are autoimmune hepatitis (AIH) types 1-3, primary biliary cirrhosis (PBC) and a hybrid form of these two diseases, the immuncholangiopathie. The AIH is a chronic progressive liver disease of unknown cause, which responds well to immunosuppressive therapy, untreated, however, the prognosis is bad. The PBC is a chronic inflammatory disease of the small and medium bile ducts. Unrecognized, it may lead to liver cirrhosis. An early and reliable diagnosis is therefore of great importance.

Antibodies against:

- AMA M2 react with the proteins of the ketoacid-dehydrogenase complex of mitochondria. They occur in 95 % of PBC patients in high titers. Their evidence is crucial for the diagnosis of PBC and for differentiation from other cholestatic liver diseases.
- the soluble core protein Sp100 are found in about 20-30 % of patients with PBC (Blüthner et al. 1999). They rarely occur in AIH (8 %) and systemic lupus erythematosus (SLE) (10 %)(Wichmann et al. 2003).
- Liver and kidney microsomes 1 (LKM1, liver kidney microsomes) and antibodies against soluble liver antigen (SLA) are typical for AIH.
- gp210 are high specific for PBC (99 %). However, they occur in only 25 % of PBC patients (Bandin et al., 1996). The gp210 antigen is an integral membrane glycoprotein of the nuclear envelope and part of the nuclear pore.
- LC1 are directed against cytosolic components of liver cells. They occur in 30 % of patients with anti-LKM-1 positive AIH, in 10 % of AIH patients they are the only circulating liver-related autoantibodies.

Principle of the test

The antigens are applied as lines on a nitrocellulose membrane. The membrane is blocked to prevent unspecific reactions. Membrane-strips with specific antigens at exactly defined positions are incubated in serum/plasma samples diluted 1:101. 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. Unbound conjugate is washed off in the following step. After the addition of the TMB-substrate it is converted by an enzymatic reaction to a blue precipitate. The reaction is stopped by distilled water.

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Blocking Reagent	3 x for 10 ml Concentrate each	white	N/A	Non-fat dry milk powder for preparation of 3 x 10 ml sample buffer
Wash Buffer (20x)	1 x 50 ml	white	colorless	20x concentrated for preparation of 1 L Tris buffer, pH 6.9 ± 0.2
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Conjugate, IgG	1 x 10 ml	blue	colorless	Containing: Anti-human immunoglobulin G (IgG) conjugated to horseradish peroxidase
TMB Substrate	1 x 10 ml	black	colorless	Stabilized TMB/H_2O_2
Membrane strips	24 strips	colour coding: brown	N/A	Coated antigens see Intended use
tweezers, reference template, scoring sheet, adhesive strip (double-sides, black)	1 pcs. each	N/A	N/A	N/A
incubation tray	3 pcs.	N/A	N/A	N/A
Labels for sample buffer	3 pcs.	N/A	N/A	N/A
MATERIALS REQUIRED, BUT NOT PROVIDED				
rocking platform, cylinder 1000 ml, pipette or cylinder for 10 ml, precision pipettes (10, 1000 µl), absorbent or filter 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 membrane-strips 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 at least six weeks. Reagents and strips shall be used within the expiry date indicated on each respective component. Don't use components after the expiry dates. Avoid intense exposure of TMB solution to the light.

5 Precautions of Use and General Introductions

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 under the conditions of 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 avoiding contact with eyes and skin and wearing disposable gloves.

Substrate contains kathon (1% v/v) as preservative. It must not be swallowed or allowed to come into contact skin or mucous membrane.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

Handle patient samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 General directions for use

To differentiate between the various **AESKUBLOT**-tests available, a color coding is applied above the reference line of the strips:

Colour coding	AESKUBLOT
yellow	ANA-12 Pro
orange	ANA-17 Pro
blue	Myositis Pro
brown	Liver Pro
purple	Vasculitis Pro
black	Gastro Pro
green	Borrelia-G and Borrelia-M

In case that the product information, including the labeling, is incorrect please contact the manufacturer or the supplier of the test kit.

Blocking Reagent and wash buffer may be interchanged between lots and test kits. All other components are specific for each test kit and are not to be interchanged. Do not exchange reagent components between autoimmunity and borrelia diagnostic tests!

For handling of conjugate do not use polystyrene vessels.

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.

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 priorly used with other reagents.

The intensity of the band colour does not necessarily correlate with antibody titers obtained by other reference methodologies.

Samples from apparent normal blood donors may contain autoantibodies.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

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/plasma 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/plasma samples should be used during the first 8 h. Alternatively, the samples should be stored in tightly closed vials at 2-8°C/35-46°F for up to 48 h, or frozen at -20°C/-4°F for longer periods. Avoid repeated thawing and freezing. Do not use heat inactivated samples.

7 Assay Procedure

7.1 Preparations prior to starting

Confirm that no salt crystals have been formed in the concentrate. If this happened, dissolve the crystals by slightly warming, room temperature should be enough, the concentrate.

Dilute concentrated wash buffer 1:20 with distilled water (e.g. 950 ml plus 50 ml).

For preparation of sample buffer: add 10 ml wash buffer to one bottle Blocking Reagent and mix well.

7.2 Test Steps

Important notes:

Follow exactly this protocol. Make sure that the two components mentioned in the protocol are added to the tray in step 2, 6, 9.

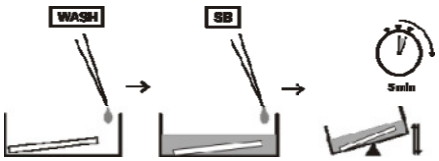
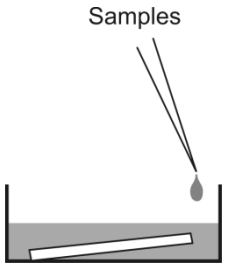
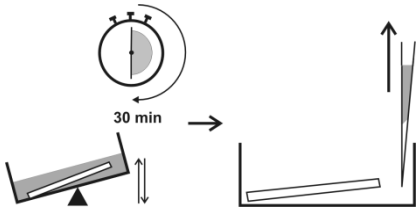
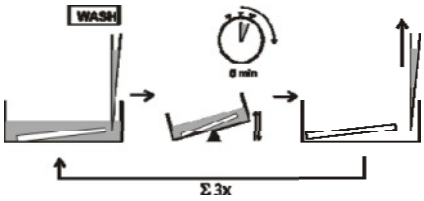
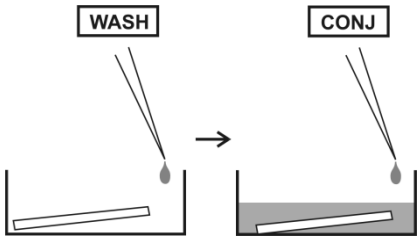
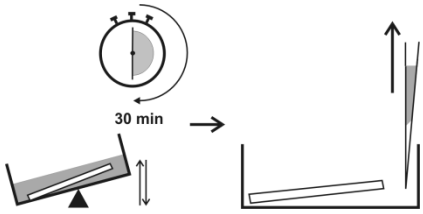
Do not let strip dry out during incubation steps.

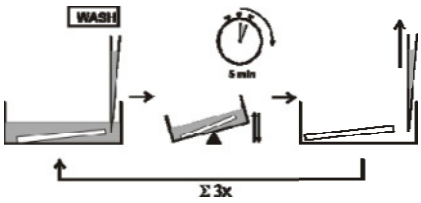
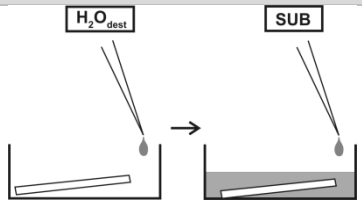
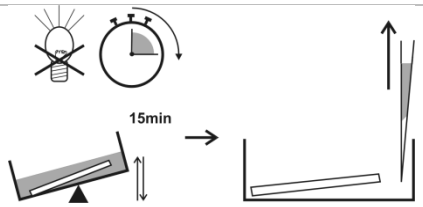
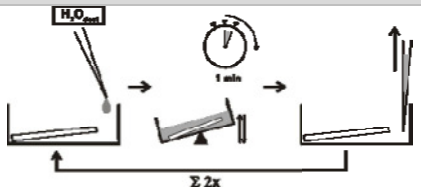
Do not touch strip with fingers, use tweezers.

Remove diluted samples completely after incubation of strip to avoid carry over.

Continuously shake strip during incubation steps.

Give sample buffer, conjugate and substrate together with the wash buffer to one side of the incubation tray. Do not allow to flow over the strip.

Step	*Description*
1.	Ensure the preparations, from step 7.1 above, have been carried out prior to test begin.
2.	 Put strip in correct orientation into incubation tray (reference line and colour coding upwards). Put 700 µl wash buffer and 300 µl sample buffer in the incubation tray. Moisten strip with the solution and incubate for 5 minutes with agitation.
CONTROLS & SAMPLES	
3.	 Pipette 10 µl serum/plasma sample into the designated incubation trays with sample buffer.
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F with agitation. After that remove sample completely.
5.	 Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.
CONJUGATE	
6.	 Pipette 700 µl wash buffer and 300 µl conjugate into each incubation tray with strip.
7.	 Incubate for 30 minutes at 20-32°C/68-89.6°F with agitation. Remove conjugate.

8.		Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.
SUBSTRATE		
9.		Pipette 700 µl dH_2O and 300 µl substrate into each incubation tray with strip.
10.		Incubate for 15 minutes at 20-32°C/68-89.6°F with agitation, protected from intense light. Remove substrate.
STOP		
11.		Pipette 2 ml dH_2O into each incubation tray with strip. Incubate 1 minute with agitation. Remove dH_2O. Repeat this step one time.
12.	Remove strip of the incubation tray. Dry strip between filter paper	
13.	Analyze results within 24 h.	

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8 Qualitative Interpretation

8.1 Manual Analysis

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Colour intensity of cut-off control is weaker than colour intensity of functional control

Fix dried strip onto scoring sheet aligned with reference line. Align reference template with the strip reference line. Interpret results only in reference to cut-off control of each strip.

Each test kit contains a colour copy with all bands provable in the test.

The analysis is carried out by means of comparing the colour intensities of the bands with colour intensity of the cut-off control. The test is equivocal if the intensities do not significant differ. Is the colour more intensive the test result is positive, if the colour intensity is weaker, the test is negative.

The results can be recorded on the scoring sheet.

In case that the values of the controls do not meet the criteria, the test is invalid and has to be repeated. We recommend retesting samples that are borderline.

The following technical issues should as well be checked: expiry date of (prepared) reagents, storage conditions, pipettes, equipment, incubation conditions and washing methods.

If the samples tested show aberrant values or any kind of deviation or if the validation criteria are not met because of reasons outside the operator's responsibility, please contact the manufacturer or the supplier of the test kit.

Medical laboratories might perform an in-house quality control by using their own controls and/or internal pooled sera, as stated in national regulations.

9 Technical Data

Sample material:	serum or plasma
Sample volume:	10 µl of sample
Total incubation time:	112 minutes at 20-32°C/68-89.6°F
Storage:	at 2-8°C/35-46°F; use original vials only.
Number of determinations:	24 tests

10 Performance Data

10.1 Relative Sensitivity and Specificity

In order to determine the positive agreement (relative sensitivity), 65 sera from IIF or ELISA antibody-positive patients were tested in **AESKUBLOT Liver Pro.** For determination of the negative agreement (relative specificity), 220 sera from blood donors were analyzed.

	positive agreement (relative sensitivity)	negative agreement (relative specificity)
AMA M2	96%	100 %
Sp100	100 %	100 %
LKM1	100 %	100 %
gp210	100 %	100 %
LC1	100 %	100 %
SLA	100 %	100 %

11 Literature

Bandin O, Courvalin J, Poupon R, Dubel L, Homberg J, Johanet C (1996). Specificity and sensitivity of gp210 autoantibodies detected using an enzyme-linked immunosorbent assay and a synthetic polypeptide in the diagnosis of primary biliary cirrhosis. Hepatology, 23: 1020–1024.

Blüthner M, Schäfer C, Schneider C, Bautz FA (1999). Identification of major linear epitopes on the sp100 nuclear PBC autoantigen by the gene-fragment phage-display technology. Autoimmunity., 29(1):33–42.

Wichmann I, Montes-Cano MA, Respaldiza N, Alvarez A, Walter K, Franco E, Sanchez-Roman J, Nún˜ez-Roldán A (2003). Clinical significance of anti-multiple nuclear dots/Sp100 autoantibodies. Scand J Gastroenterol. 38(9):996–9

For further reading:

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

Kanzler S, Weidemann C, Gerken G, Löhr H, Galle PR, Meyer zum Büschenfelde KH, Lohse AW (1999). Clinical significance of autoantibodies to soluble liver antigen in autoimmune hepatitis. J Hepatol 31: 635–640.

Krawitt EL (1996). Autoimmune Hepatitis. N Engl J Med 334: 897–903.

Manns MP, Griffin KJ, Sullivan KF, Johnson EF (1991). LKM-1 autoantibodies recognize a short linear sequence in P450 IID6, a cytochrome P-450 monooxygenase. J Clin Invest 88: 1370–1378.

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

Wies I, Brunner S, Henninger J, Herkel J, Kanzler S, Meyer zum Büschenfelde KH, Lohse AW (2000). Identification of target antigen for SLA/LP autoantibodies in autoimmune hepatitis. Lancet 355: 1510–1515.

IVD	¨ Diagnosi in vitro	¨ For in vitro diagnostic use
	¨ Pour diagnostic in vitro	¨ Para uso diagnóstico in vitro
	¨ In Vitro Diagnostikum	¨ In Vitro Διαγνωστικό μέσο
	¨ Para uso Diagnóstico in vitro	
REF	¨ Numero d'ordine	¨ Cataloge number
	¨ Référence Catalogue	¨ Numéro de catálogo
	¨ Bestellnummer	¨ Αριθμός παραγγελίας
	¨ Número de catálogo	
LOT	¨ Descrizione lotto	¨ Lot
	¨ Lot	¨ Lote
	¨ Chargen Bezeichnung	¨ Χαρακτηρισμός παρτίδας
	¨ Lote	
CE	¨ Conformità europea	¨ EC Declaration of Conformity
	¨ Déclaration CE de Conformité	¨ Declaración CE de Conformidad
	¨ Europäische Konformität	¨ Ευρωπαϊκή συμφωνία
	¨ Déclaracão CE de Conformidade	
Σ 24	¨ 24 determinazioni	¨ 24 tests
	¨ 24 tests	¨ 24 pruebas
	¨ 24 Bestimmungen	¨ 24 προσδιορισμοί
	¨ 24 Testes	
i	¨ Rispettare le istruzioni per l'uso	¨ See instructions for use
	¨ Voir les instructions d'utilisation	¨ Ver las instrucciones de uso
	¨ Gebrauchsanweisung beachten	¨ Λάβετε υπόψη τις οδηγίες χρήσης
	¨ Ver as instrucões de uso	
	¨ Da utilizzarsi entro	¨ Use by
	¨ Utilise avant le	¨ Utilizar antes de
	¨ Verwendbar bis	¨ Χρήση μέχρι
	¨ Utilizar antes de	
+2°C +8°C	¨ Conservare a 2-8°C	¨ Store at 2-8°C (35-46°F)
	¨ Conserver à 2-8°C	¨ Conservar a 2-8°C
	¨ Lagerung bei 2-8°C	¨ Φυλάσσεται στους 2-8°C
	¨ Conservar entre 2-8°C	
	¨ Prodotto da	¨ Manufactured by
	¨ Fabriqué par	¨ Fabricado por
	¨ Hergestellt von	¨ Κατασκευάζεται από
	¨ Fabricado por	
STRIP	¨ Strip di nitrocelluslosa rivestita	¨ Coated nitrocellulose strip
	¨ Strip de nitrocellulose couché	¨ Tira de nitrocelulosa recubierta
	¨ Nitrozellulosemembran-Streifen mit aufgebrachten Antigenen	¨ Επίστρωση λωρίδα νιτροκυτταρίνης
	¨ Tira de nitrocelulose revestido	
WASH 20x	¨ Tampone di lavaggio	¨ Wash buffer
	¨ Tampon de Lavage	¨ Solución de lavado
	¨ Waschpuffer	¨ Ρυθμιστικό διάλυμα πλύσης
	¨ Solucão de lavagem	
Block-Reag	¨ Reagente bloccante	¨ Blocking Reagent
	¨ réactif de blocage	¨ Reactivo bloqueante
	¨ Blockier-Reagenz	¨ Αντιδραστήριο αποκλεισμού
	¨ Bloqueio de reagente	¨
RCNS 10ml	¨ Ricostituire con 10 mL	¨ Reconstitute with 10 mL
	¨ reconstituer avec 10 mL	¨ reconstituir con 10 mL
	¨ rekonstituiren mit 10 mL	¨ Ανασύσταση με 10 mL
	¨ reconstituir com 10 mL	¨
SB	¨ Tampone campione	¨ Sample buffer
	¨ Tampon Echantillons	¨ Tampón Muestras
	¨ Probenpuffer	¨ Ρυθμιστικό διάλυμα δειγμάτων
	¨ Diluente de amostra	
CONJ	¨ Coniugato	¨ Conjugate
	¨ Conjugé	¨ Conjugado
	¨ Konjugat	¨ Σύζευγμα
	¨ Conjugado	
SUB	¨ Tampone substrato	¨ Substrate buffer
	¨ Substrat	¨ Tampón sustrato
	¨ Substratpuffer	¨ Ρυθμιστικό διάλυμα υποστρώματος
	¨ Substrato	