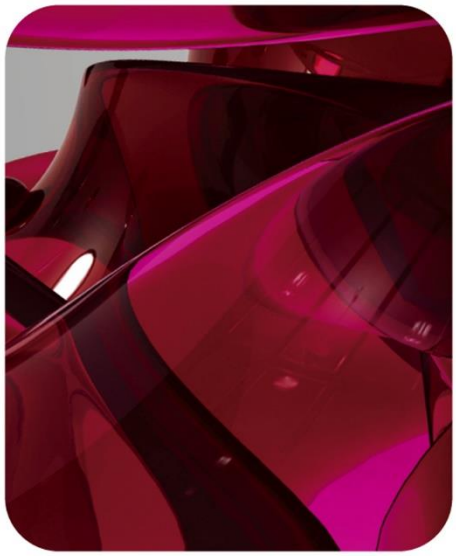
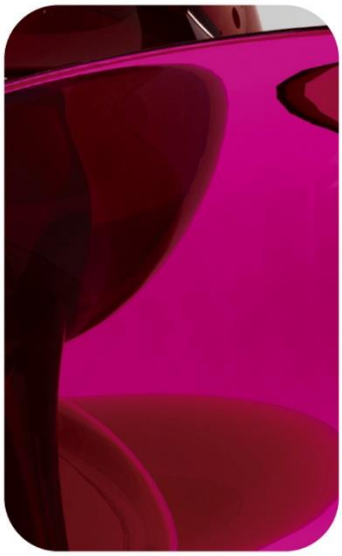
AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS


AESKUBLOTS®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKUBLOTS® Liver Pro

Ref 4004

DIN EN ISO 13485

Instruction Manual

Table of Contents

Aesku.Diagnostics GmbH & Co. KG
Mikroforum Ring 2
55234 Wendelsheim, Germany
Tel: +49-6734-9622-0
Fax: +49-6734-9622-2222
Info@aesku.com
www.aesku.com

1 Intended Use

***AESKUBLOTS*® 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. 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 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 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	color coding: brown	N/A	Coated antigens see Intended use
tweezers, reference template, scoring sheet, adhesive strip (double-sides, white)	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/35.6-46.4°F in their original containers. Once prepared, reconstituted wash buffer as well as opened strips, conjugate and TMB are stable at 2-8°C/35.6-46.4°F for at least six weeks. Reconstituted blocking reagent is stable at 2-8°C/35.6-46.4°F for at least 3 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.

All kit components are classified according to Regulation (EC) No. 1272/2008 [CLP]. See the Materials and Safety Document (MSDS) for more information on ingredients.

Substances listed on the so-called "Candidate List of Substances of very High Concern (SVHCV) for authorization" of the European Chemicals Agency (ECHA) are not intentional components of this product. It is therefore not to be expected that these substances are contained in amounts ≥ 0.1% in the product.

Reagents should be stored safely and be inaccessible to children.

In particular, the mixture does not contain any substances in concentrations ≥ 0.1 % to be classified as PBT or vPvB.

Patient samples should be considered potentially infectious and handled according to national laws. Patient samples and other potentially infectious material should be decontaminated after the test run.

5.2 General directions for use

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

Color coding	***AESKUBLOTS***®
red	ANA-17 comp
orange	ANA-17 Pro
blue	Myositis Pro
brown	Liver Pro
purple	Vasculitis Pro
black	Gastro Pro
green	Borrelia-G and Borrelia-M

In case 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 color 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 samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolyzed 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. Alternatively, the samples should be stored in tightly closed vials at 2-8°C/35.6-46.4°F for up to 48 h, or frozen at -20°C/-4°F for longer periods (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4 Vol. 30 No. 10). Avoid repeated thawing and freezing. Do not use heat inactivated samples (56°C/132.8°F).

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. 50 ml plus 950 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.

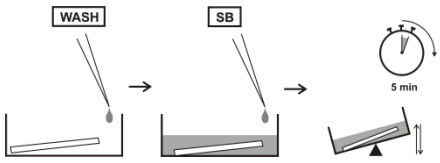
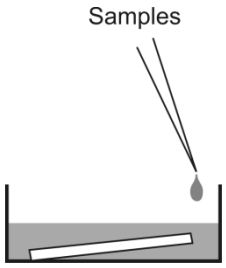
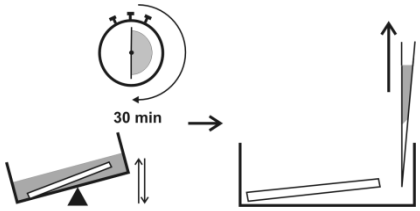
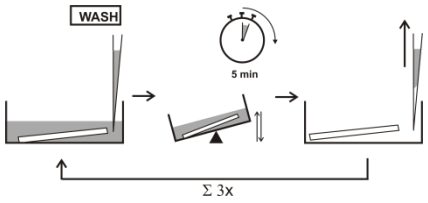
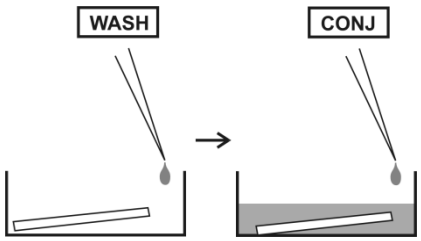
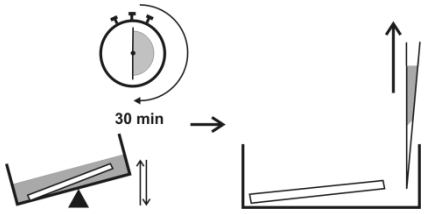
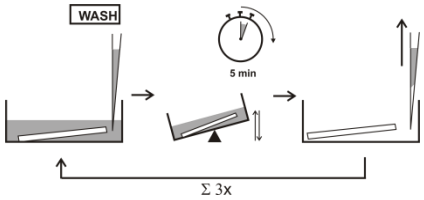
Do not let strip dry out during incubation steps.

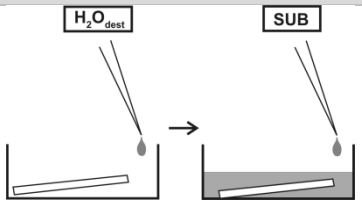
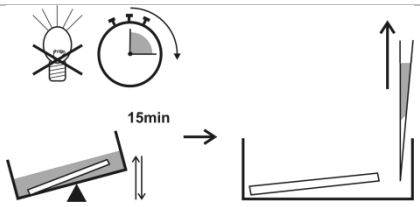
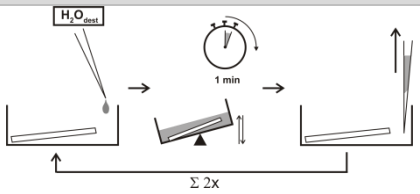
Do not touch strip with fingers, use tweezers.

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 color 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 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.	

AESKUBLOTS® Liver Pro is also intended to be automatically processed and evaluated on the HELIA® Automated blot system.
Reagent preparation for HELIA®: Dilute one part wash buffer concentrate (WASH) with 19 parts ultrapure water (e.g. 50 ml wash buffer concentrate and 950 ml ultrapure water) to obtain a ready-to-use wash buffer. All other reagents are ready to use when processed in HELIA®. For detailed handling of the test on HELIA® refer to the instruction manual of the HELIA®.

.

8 Qualitative Interpretation

8.1 Manual Analysis

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Color intensity of cut-off control is weaker than color 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 color copy with all bands provable in the test.

The analysis is carried out by means of comparing the color intensities of the bands with color intensity of the cut-off control. The test is equivocal if the intensities do not significant differ If the color is more intense, the test result is positive, if the color 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.

8.2 Software-supported evaluation

The analysis of the strips can be carried out by means of using AESKU.SCAN Software. Please refer to the instructions for use of AESKU.SCAN.

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Color intensity of cut-off control is weaker than color intensity of functional control

<u>AESKU.SCAN 2.0:</u>

Fix dried strip onto scoring sheet (printable) aligned with reference line. Align reference template with the strip reference line.

Evaluate strips according to the instructions for use of AESKU.SCAN 2.0 software.

Qualitative result analysis is carried out by means of comparing the color intensities of the individual antigens with the color intensity of the cut-off control.

AESKU.SCAN 3.0:

Put strips within the incubation tray into the reader.

Evaluate strips according to the instructions for use of AESKU.SCAN 3.0 software.

Qualitative result analysis is carried out by means of comparing the color intensities of the individual antigens with the color intensity of the cut-off control.

HELIA®:

Using a HELIA® line immune assay analyzer, the results are analyzed automatically. The results can be determined in Index-values.

The following interpretation according to the signal intensity is suggested:

Result Interpretation	Symbol	Index	Color
Negative	-	0.0 - <0.8	Colorless
Equivocal	+/-	≥0.8 - <1.15	Blue
Weak positive	+	≥1.15 - <2.5	Yellow
Positive	++	≥2.5 - <4.0	Red
Strong positive	+++	≥ 4.0	Dark red

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
Sample volume:	10 µl of sample
Total incubation time:	112 minutes at 20-32°C/68-89.6°F
Storage:	at 2-8°C/35.6-46.4°F; use original vials only.
Number of determinations:	24 tests

10 Performance Data

Normal Range Study

Expected values for AESKUBLOTS® Liver Pro were analyzed with a panel of 120 sera from healthy donors.

All assays were performed fully automated according to the current IFUs.

AESKUBLOTS® Liver Pro – Normal Range										
Antigen	Number of samples	Positive samples		Equivocal samples		Negative samples		Min [Index]	Max [Index]	Mean [Index]
		n	(%)	n	(5(	n	(%)			
AMA M2	120	1	0.83	1	0.83	118	98.33	0.00	1.26	0.15
Sp100	120	0	0.00	0	0.00	120	100.00	0.00	0.16	0.01
LKM1	120	0	0.00	0	0.00	120	100.00	0.00	0.27	0.01
Gp210	120	0	0.00	0	0.00	120	100.00	0.00	0.68	0.13
LC1	120	0	0.00	0	0.00	120	100.00	0.00	0.36	0.06
SLA	120	0	0.00	0	0.00	120	100.00	0.00	0.24	0.01

With AESKUBLOTS® Liver Pro there was one positive and one equivocal result for anti-AMA M2. Lowest Index-value measured was 0.0, highest Index-value measured was 1.26 (AMA M2).

The low number of positive samples we found with AESKUBLOTS® Liver Pro in the tested healthy population correlates well with the numbers reported in the literature.

We also recommend in the IFU in chapter 8: “Quantitative and qualitative interpretation” that each laboratory should establish its own normal range.

Precision

The precision of the test results obtained with the AESKUBLOTS® Liver Pro was investigated by determining the intra- and inter-assay precision as well as the lot variance by analyzing several samples.

Intra-Assay Precision

To determine the within-lab / intra-assay variances five different sera (S1-S4) have been tested for 20 times on AESKUBLOTS® Liver Pro. The positive- and negative agreement were calculated from these test values (n=20).

Calculation of agreements	
Percent positive-agreement [%]	100.0
Percent negative-agreement [%]	100.0
Percent overall-agreement [%]	100.0

Inter-Assay Precision

To determine the inter-assay / day to day variances four different sera (S1-S4) over the whole range have been tested 8 times per run, in a total of 5 runs on 5 different days (n=40). For day 1 the results from the within-lab / intra-assay variances are used (n=20), the positive- and negative agreement were calculated from these test values.

Calculation of agreements	
Percent positive-agreement [%]	100.0
Percent negative-agreement [%]	100.0
Percent overall-agreement [%]	100.0

Lot to Lot Variation

To determine the Lot to Lot variation four different sera (S1-S4) over the whole range have been tested 8 times on 3 Lots (n=24). Lot 1 is taken from Run 1 of the Intra Assay data. For each sample the variation coefficients and the mean values have been calculated across the test values of the three different lots, positive- and negative agreement were calculated from these test values.

Calculation of agreements	
Percent positive-agreement [%]	100.0
Percent negative-agreement [%]	100.0
Percent overall-agreement [%]	100.0

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 ***AESKUBLOTS***® **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 %

Diagnostic Sensitivity and Specificity

A clinical study with different serum samples from patients with primary biliary cholangitis (PBC), Hepatitis-B virus (HBV), Hepatitis-C virus (HCV) and healthy controls were analyzed with the AESKUBLOTS® Liver Pro. Furthermore, these samples were measured with a competitor Western blot. All assays were performed according to the current IFUs. After that the results were compared to determine the sensitivity* and specificity* of the assay.

	Sensitivity	Specificity HCV/ HBV patients	Specificity healthy controls
AMA M2	89.7%	98.9%	100.0%
Sp100	85.7%	100.0%	100.0%
gp210	85.7%	99.3%	100.0%
LKM1	n.A.*	100.0%	100.0%
SLA	n.A.*	100.0%	100.0%
LC1	n.A.*	100.0%	100.0%

Sensitivities could not be calculated for anti-LKM1, anti-SLA and anti-LC1 due to lack of samples. For anti-AMA M2, anti-Sp100 and anti-gp210 sensitivity > 85% could be calculated. The specificities of HCV and HBV patients and healthy controls showed very good results of > 98%. Due to the lack of positive samples for autoimmune hepatitis further investigations need to be done.

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, Núñez-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	
	¨ 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 (35.6-46.4°F)	¨ Store at 2-8°C (35.6-46.4°F)
	¨ Conserver à 2-8°C (35.6-46.4°F)	¨ Conservar a 2-8°C (35.6-46.4°F)
	¨ Lagerung bei 2-8°C (35.6-46.4°F)	¨ Φυλάσσεται στους 2-8°C (35.6-46.4°F)
	¨ Conservar entre 2-8°C (35.6-46.4°F)	
	¨ 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	¨
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	