AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS



AESKULISA®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA® APS-Profile-GM

Ref 3234

CE | Σ 96 | 2°C – 8°C | IVD | eIFU i WWW.AESKU.COM

Instruction Manual

Table of Contents

1 Intended Use 1
2 Clinical Application and Principle of the Assay 1
3 Kit Contents 2
4 Storage and Shelf Life 2
5 Precautions of Use 3
6 Sample Collection, Handling and Storage 4
7 Assay Procedure 4
8 Qualitative Interpretation 7
9 Technical Data 8
10 Performance Data 8
11 Literature 9
12 Regulatory Symbols 10

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

1 Intended Use

***AESKULISA®* APS-Profile-GM** is a solid phase enzyme immunoassay for the separate qualitative detection of IgG and/or IgM antibodies against phospholipids in human serum. The assay employs highly purified human Prothrombin (Factor II), Thrombin, Cardiolipin, Phosphatidyl-choline, -ethanolamine, -inositol, -serine and Sphingomyelin.

The assay is an aid in the diagnosis and risk estimation of thrombosis in patients with systemic lupus erythematosus.

2 Clinical Application and Principle of the Assay

Antibodies against phospholipids, components of the biological membranes, are specific for phospholipids such as Cardiolipin, Phosphatidyl -inositol, -ethanolamine, -choline and Sphingomyelin.

Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases. The occurrence of anti-phospholipid antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-phospholipid antibodies in patients with no other autoimmune diseases characterize the primary APS.

Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of placental infarct). The exact mechanisms by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

Antibodies targeting prothrombin alone have recently been described being highly associated with fetal loss in APS patients. Prothrombin antibodies are the the first marker for this serious complication as all other known antiphospholipid antibodies failed to correlate with fetal loss.

The presence of anti-thrombin antibodies has been associated with the clinical features of the antiphospholipid syndrome (APS). Anti-Thrombin antibodies seem to correlate with deep venous thrombosis.

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
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	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
Negative Control	2 x 1.8ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	8 x 1.5ml	Blue	Yellow	Antigen specific Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG IgM	1 x 15ml 1 x 15ml	Blue Green	Blue Green	Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
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/35.6-46.4 °F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8 °C/35.6-46.4 °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 8h, respectively stored tightly closed at 2-8 °C/35.6-46.4 °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/35.6-46.4 °F).

7.2 Pipetting Scheme

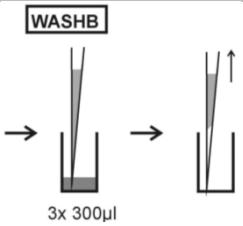
We suggest pipetting calibrators, controls and samples as follows:

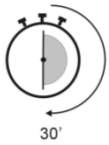
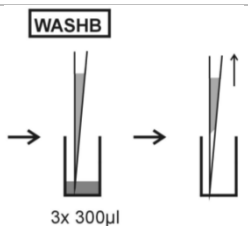
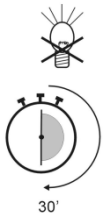
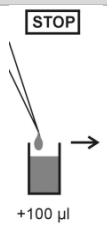
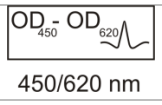
NOTE: If IgG and IgM are determined in parallel, calibrators, controls and samples have to be done for each subclass separately.

Antigen		1	2	3	4...	
Prothrombin	A	CCA	NC	P1	P2	P3
Thrombin	B	CCB	NC	P1	P2	P3
Cardiolipin	C	CCC	NC	P1	P2	P3
P.-cholin	D	CCD	NC	P1	P2	P3
P.-ethanolamin	E	CCE	NC	P1	P2	P3
P.-inositol	F	CCF	NC	P1	P2	P3
P.-serine	G	CCG	NC	P1	P2	P3
Sphingomyelin	H	CCH	NC	P1	P2	P3

PC: positive control
NC: negative control
CCA-CCH: 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: a. Cut-off Calibrator (CCA-CCH) for *QUALITATIVE* interp. and 100 µl of each of the following: • Negative control (NC) 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 Qualitative Interpretation

Read the optical density of the specific calibrator (A-H) and the patient samples. Multiply the OD of the calibrator by the parameterspecific factor, provided with the lot specific QC certificate. 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

APS Profile	O.D. 450/620 nm
Negative Control	0.033
Cut-off Calibrator	0.5225

Example of interpretation

We recommend pipetting cut-off calibrator in parallel for each run.

Measured: ODCut-off Calibrator (Cardiolipin): 0.5225

Negative:	$OD_{Patient}$	< 0.8 x $OD_{Cut\text{-}off\ Parameter}$	= 0.8 x 0.5225	=0.418
Positive:	$OD_{Patient}$	> 1.2 x $OD_{Cut\text{-}off\ Parameter}$	= 1.2 x 0.5225	=0.627
Equivocal:	0.418 ≤	$OD_{Patient}$		≤ 0.627

ID Nr.	Sample	OD - Calculation	Interpretation
	OD Cardiolipin		
1	0.99	> 0.627	--->Positive
2	0.49	≥ 0.418 und ≤ 0.627	--->Equivocal
3	0.27	< 0.418	--->Negative

Do not use this example for interpreting patients results!

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 EU 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 semi-quantification of the results, each patient-OD value can be expressed by the Index-Value. The Index-Value is calculated by dividing the patient-OD by the cut-off parameter:

$$\textbf{Index Value} = \frac{\textbf{OD (patient sample)}}{\textbf{OD (cut-off calibrator)}}$$

Negative:		**Index Value**	**< 0.8**
Equivocal:	**0.8≤**	**Index Value**	**≤ 1.2**
Positive:		**Index Value**	**>1.2**

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
Storage:	at 2-8 °C/35.6-46.4 °F use original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Specificity and sensitivity

The microplate is coated with Prothrombin, Thrombin, Cardiolipin, Phophatidylcholine, -ethanolamine, -inostol, -serine and Sphingomyelin. No crossreactivities to other autoantigens have been found. Up to 50 % of pregnant women with APS suffer from fetal loss. Prothrombin identifies 84 % of those. Since ***AESKULISA®*** **APS-Profile-GM** consists of various antigens, the known values for IgG sensitivity and specificity are listed in the table below.

	Sensitivity	Specificity
Cardiolipin	67%	73%
Phosphatidylserine	62%	83%
Phosphatidyl-Inositol	69%	75%
Ethanolamine	62%	78%
Choline	62%	79%

10.2 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 concentration (OD-Ratio)	expected concentration (OD-Ratio)	Recovery (%)
1	1 / 100	7.40	7.50	98.7
	1 / 200	3.50	3.75	93.3
	1 / 400	1.75	1.88	93.1
	1 / 800	0.88	0.94	93.6
2	1 / 100	3.60	3.50	102.9
	1 / 200	1.71	1.75	97.7
	1 / 400	0.85	0.88	96.6
	1 / 800	0.43	0.44	97.7

10.3 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			Inter-assay		
APS Profile	**Mean OD-Ratio**	**CV (%)**	**APS Profile**	**Mean OD-Ratio**	**CV (%)**
Prothrombin	2.4	3.1	Prothrombin	2.8	2.8
Thrombin	1.8	2.8	Thrombin	1.5	3.1
Cardiolipin	1.5	1.5	Cardiolipin	1.3	3.6
Phosphatidyl-Choline	3.2	1.6	Phosphatidyl-Choline	2.6	2.1
Phosphatidyl-Ethanolamine	3.5	1.9	Phosphatidyl-Ethanolamine	2.9	3.5
Phosphatidyl-Inositol	3.1	2.2	Phosphatidyl-Inositol	4.1	2.6
Phosphatidyl-Serine	2.6	2.9	Phosphatidyl-Serine	3.8	1.5
Sphingomyelin	2.1	3.1	Sphingomyelin	3.6	2.4

11 Literature

Boey, M.L., Colaco, C.B., Gharavi, A.E., et al. (1983): Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. Br. Med. J. 287: 1021-1023.

Gastineau, D.A., Kazmier, F.J., Nichols, W.L., Bowie, E.J. (1985): Lupus anticoagulant: an analysis of the clinical and laboratory features of 219 cases. Am. J. Hematol. 19: 265-267.

McNeil HP, Simpson RJ, Chesterman CN, Kirilis SA (1990): Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: ß2-Glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 87: 4120-4124.

Wöhrle R, Matthias T, von Landenberg P, Oppermann M, Helmke K, Förger F (2000): Clinical relevance of antibodies against different phospholipids. Journal of Autoimmunity 15: A60.

Wöhrle R, Matthias T, von Landenberg P, Oppermann M, Förger F, Helmke K (2000): A new anti.phospholipid-antibody ELISA - sensitivity and specificity for cerebrovascular insults in autoimmune diseases. Journal of Autoimmunity Vol 15: A 60.

12 Regulatory Symbols

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	
UDI	- Identificatore univoco del dispositivo	- Unique Device Identifier
	- Identifiant unique de l'appareil	- Identificador único del dispositivo
	- eindeutige Produktidentifizierung	- Μοναδικό αναγνωριστικό συσκευής
	- Identificador único do dispositivo	
CE	- Conformità europea	- EC Declaration of Conformity
	- Déclaration CE de Conformité	- Declaración CE de Conformidad
	- Europäische Konformität	- Ευρωπαϊκή συμφωνία
	- Déclaracão CE de Conformidade	
Σ 96	- 96 determinazioni	- 96 tests
	- 96 tests	- 96 pruebas
	- 96 Bestimmungen	- 96 προσδιορισμοί
	- 96 Testes	
eIFU WWW.AESKU.COM	- Rispettare le istruzioni elettroniche per l'uso	- See electronical instructions for use
	- Voir les instructions d'utilisation électronique	- Siga las instrucciones electrónicas de uso
	- Elektronische Gebrauchsanweisung beachten	- Ακολουθήστε τις ηλεκτρονικές οδηγίες χρήσης
	- Seguir as instruções electrónicas de utilização	
	- Da utilizzarsi entro	- Use by
	- Utilise avant le	- Utilizar antes de
	- Verwendbar bis	- Χρήση μέχρι
	- Utilizar antes de	
+8°C +2°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	
CO-CAL	- Calibratore cut-off	- Cut off Calibrator
	- Etalon Seuil	- Calibrador de cut-off
	- Grenzwert Kalibrator	- Οριακός ορός Αντιδραστήριο βαθμονόμησης
	- Calibrador de cut-off	
CON –	- Controllo negativo	- Negative Control
	- Contrôle Négatif	- Control Negativo
	- Negativ Kontrolle	- Αρνητικός ορός ελέγχου
	- Controlo negativo	
CONJ	- Coniugato	- Conjugate
	- Conjugé	- Conjugado
	- Konjugat	- Σύζευγμα
	- Conjugado	
MP	- Micropiastra rivestita	- Coated microtiter plate
	- Microplaque sensibilisée	- Microplaca sensibilizada
	- Beschichtete Mikrotiterplatte	- Επικαλυμμένη μικροπλάκα
	- Microplaca revestida	
WASHB 50x	- Tampone di lavaggio	- Wash buffer
	- Tampon de Lavage	- Solución de lavado
	- Waschpuffer	- Ρυθμιστικό διάλυμα πλύσης
	- Solucão de lavagem	
SUB	- Tampone substrato	- Substrate buffer
	- Substrat	- Tampón sustrato
	- Substratpuffer	- Ρυθμιστικό διάλυμα υποστρώματος
	- Substrato	
STOP	- Reagente bloccante	- Stop solution
	- Solution d'Arrêt	- Solución de parada
	- Stopreagenz	- Αντιδραστήριο διακοπής αντίδρασης
	- Solucão de paragem	
SB 5x	- Tampone campione	- Sample buffer
	- Tampon Echantillons	- Tampón Muestras
	- Probenpuffer	- Ρυθμιστικό διάλυμα δειγμάτων
	- Diluente de amostra	