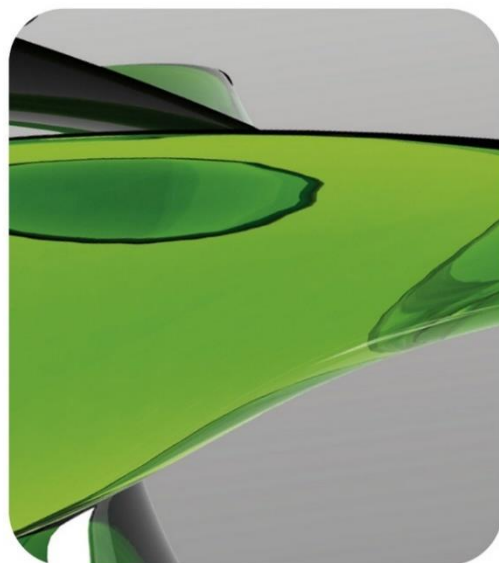




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AESKULISA[®]

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA[®] SS-A

REF 7107US



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.....	3
7	Assay Procedure.....	4
8	Quantitative and Qualitative Interpretation	5
9	Technical Data	6
10	Performance Data.....	6
11	Literature.....	7
12	Regulatory Symbols.....	8
	Annex	9



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1 Intended Use

AESKULISA® SS-A is a solid phase enzyme immunoassay for the semi-quantitative and qualitative detection of antibodies against Ro/ SS-A in human serum. The assay employs human Ro/SS-A antigen composed of purified native 60 kDa and recombinant human 52 kDa Ro/SS-A protein. Anti-SS-A antibodies preferentially react with the native 60 kDa molecule whereas most antibodies to the 52 kDa protein prefer the denatured molecule. The assay is an aid in the diagnosis of Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) and should be used in conjunction with other serological tests and clinical findings.

2 Clinical Application and Principle of the Assay

SS-A is a RNA protein particle (ribonucleoprotein, RNP) ubiquitously distributed in all tissues. It is composed of two proteins (60 kDa and 52 kDa) associated with at least four small uridin-rich cytoplasmic RNAs (hyRNA, **human cytoplasmic RNA**), its function is yet unknown.

Autoantibodies against SS-A (formerly named Ro after prototype patient Robert) as well as SS-B (formerly named La after prototype patient Lane) are typical markers for Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE), both are systemic autoimmune diseases of unknown etiology and female predominance. Sjögren's syndrome is a disorder affecting exocrine glands such as lacrimal and salivary glands. Chronic inflammation dominated by plasmacells results in a proceeding loss of function of these glands, described as Sicca-syndrome. The diagnosis of SS is based on testing for loss of excretory function in eye and salivary glands and the detection of anti-SS-A and anti-SS-B antibodies.

Antibodies against both 60 kDa and 52 kDa SS-A proteins are found in 70-80% of patients with primary SS, 40-94 % of these patients display antibodies against the SS-B/La antigen, additionally. SS-A antibodies occur in 25-40% of patients with ANA-positive SLE (ANA: anti-nuclear antibodies), as well as isolated in 65% of the patients with subacute cutaneous LE. Both, anti-SS-A and anti-SS-B antibodies are associated with congenital heartblock.

Isolated detection of 52 kDa SS-A antibodies, without 60 kDa antigen, is found more often in SS, as well as in ANA-negative LE and with a frequency of 95% in neonatal LE.

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 rate 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:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Cut-off Control 1 vial, 1.5 ml (capped blue: yellow solution)
Containing: Human serum (diluted), sodium Azide (preservative)

Calibrators 6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml
(color increasing with concentration)
Containing: Human serum (diluted), sodium azide (preservative)

Conjugate 1 vial, 15 ml IgG (capped blue: blue solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H₂O₂

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (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 and the European Pharmacopeia.

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 for 1 month at 2-8°C/35.6-46.4°F, at least.

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 normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though the kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

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.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-26°C/68-78.8°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 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 and medicinal methods if the patient has got infectious diseases accompanied by medication.

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 immediately, respectively stored tightly closed at 2-8°C/35.6-46.4°F up to three days, or frozen at -20°C/-4°F for longer periods.

7 Assay Procedure

7.1 Preparations prior to pipetting

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).

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 Work flow

- Pipette 100 µl of each patient's diluted serum into the designated microwells.
- Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-26°C/68-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/68-78.8°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 15 minutes at room temperature (20-26°C/68-78.8°F), in the dark.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8 Quantitative and Qualitative Interpretation

For the **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	Positive Results
≤ 15 U/ml	> 15 U/ml

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgG	OD 450/620 nm	CV %
0 U/ml	0.021	1.9
3 U/ml	0.153	1.8
10 U/ml	0.326	1.7
30 U/ml	0.670	2.2
100 U/ml	1.373	0.3
300 U/ml	2.239	0.5

Example of a calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.979/0.969	0.974	54.6
P 02	0.575/0.583	0.579	23.4

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. **Do not use this example for interpreting patients results!**

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For **qualitative interpretation** read the optical density of the cut-off control and the patient samples. Compare patient's OD with the OD of the cut-off control. All samples which are higher than cut-off are considered positive.

Negative:	OD patient < OD _{cut-off}
Positive:	OD patient > 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: 60 minutes at room temperature (20-26°C/68-78.8°F)
Calibration range: 0-300 U/ml
Analytical sensitivity: 1.0 U/ml
Storage: at 2-8°C/35.6-46.4°F use original vials, only
Number of determinations: 96 tests

10 Performance Data

10.1 Analytical Sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity and sensitivity

The microplates are coated with **native human 60 kDa SS-A** and **recombinant human 52 kDa SS-A**. No cross reactivities to other autoantigens have been found. Antibodies against SS-A occur in 80% of patients with Sjögren's syndrome and 40% of ANA (anti-nuclear antibodies) positive systemic lupus erythematosus patients. A study with 51 Anti-SS-A positive and 35 negative sera (from patients with various rheumatic disorders) and the **AESKULISA® SS-A** is shown in the table below.

		results from the AESKULISA SS-A	
clinical data for SS-A		positive	negative
	positive	50	1
	negative	0	35

98.8% agreement

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 measured Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%)
1	1 / 100	18.7	19.3	96.6
	1 / 200	9.6	9.7	99.0
	1 / 400	4.9	4.8	102.0
	1 / 800	2.4	2.4	100.0
2	1 / 100	61.4	58.7	105.0
	1 / 200	27.1	29.4	92.2
	1 / 400	13.7	14.7	93.2
	1 / 800	6.9	7.3	94.5



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			Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)	Sample No.	Mean (U/ml)	CV (%)
1	78.2	8.1	1	102.4	3.8
2	44.3	2.8	2	62.5	5.5
3	22.9	2.0	3	26.3	1.9

10.5 Calibration

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

11 Literature

- Itoh Y and Reichlin M (1992).**
Autoantibodies to the Ro/SS-A autoantigen are conformation dependent. Anti-60 kDa antibodies are mainly directed to the native protein; anti-52 kDa antibodies are mainly directed to the denatured protein.
Autoimmunity; 14: 57-65.
- Kalden JR (1988).**
Sjögren-Syndrom.
In Kalden JR (Hersg), Klinische Rheumatologie, S. 374-379; Springer-Verlag, Berlin.
- Harley JB (1998).**
Autoantibodies in Sjögren`s syndrome.
J. Autoimmun 2: 383-394.
- Slobbe RL, Pruijn GJM, Damen WGM et al. (1991).**
Detection and occurrence of the 60 kDa and 52 kDa (Ro (SS-A) antigens and of autoantibodies against these proteins.
Clin Exp Immunol 86: 99-105.



12 Regulatory Symbols

IVD	For in vitro diagnostic use
REF	Catalog number
LOT	Lot
UDI	Unique Device Identifier
	96 tests
	See instructions for use
	Use by
	Store at 2-8°C (35.6-46.4°F)
	Manufactured by
CON +	Positive Control
CON -	Negative Control
CAL	Calibrator
CO-CAL	Cut off Calibrator
CONJ	Conjugate
MP	Coated microtiter plate
WASHB 50x	Wash buffer
SUB	Substrate buffer
STOP	Stop solution
SB 5x	Sample buffer
Rx only	For Prescription Use only

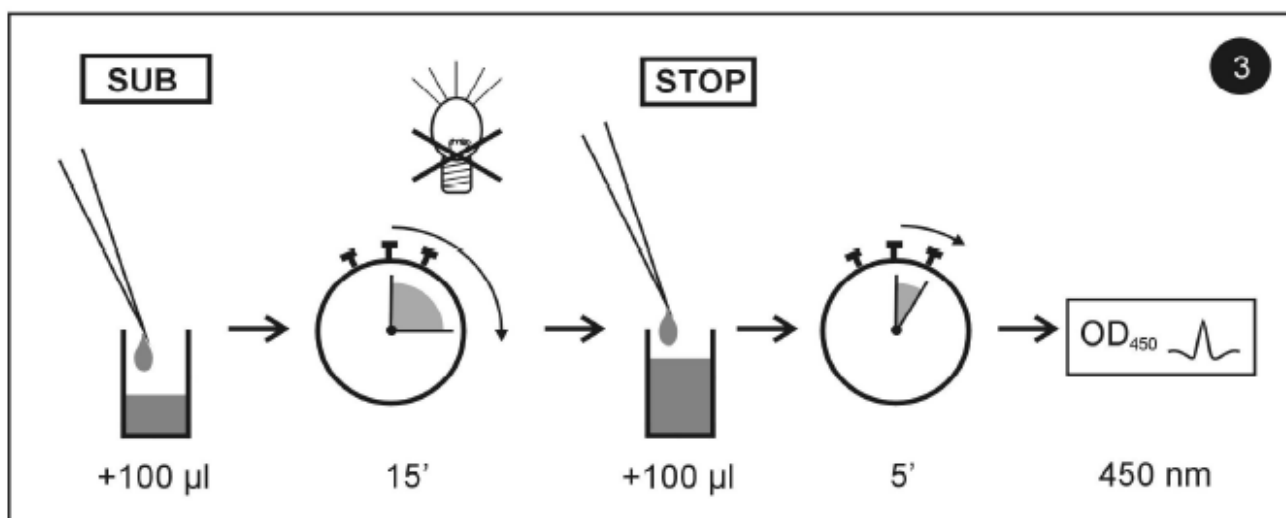
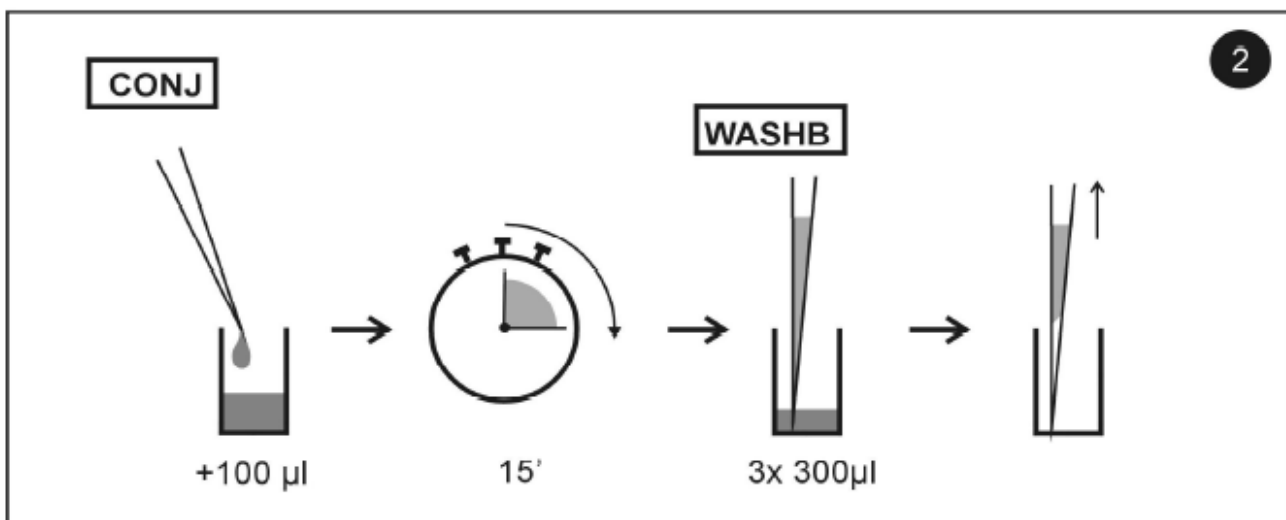
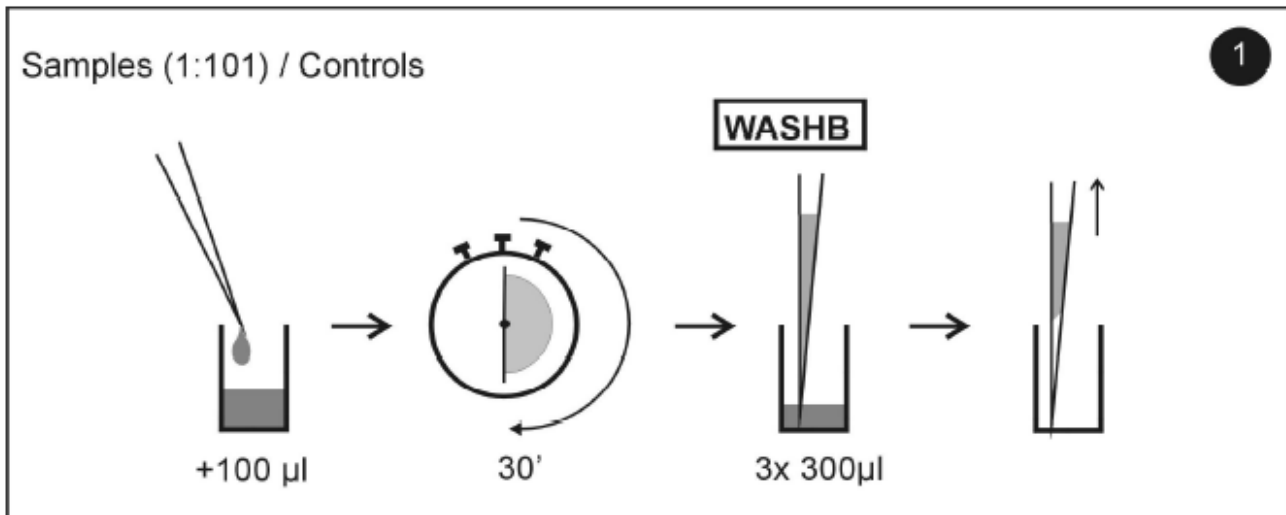
Annex

Annex A: Pipetting Scheme

Calibrators A-F				Controls	Samples
Pipette	Calibrators A-F	100 µl each	-	-	
Pipette	Controls	-	100 µl each	-	
Pipette	Prediluted samples (1:101)	-	-	100 µl each	
Incubate	30 min at room temperature (20-26°C/68-78.8°F)				
Decant	Wash 3x with 300 µl of wash buffer (1x)				
Pipette	Conjugate	100 µl	100 µl	100 µl	
Incubate	15 min at room temperature (20-26°C/68-78.8°F)				
Decant	Wash 3x with 300 µl of wash buffer (1x)				
Pipette	Substrate	100 µl	100 µl	100 µl	
Incubate	15 min at room temperature (20-26°C/68-78.8°F), in the dark.				
Pipette	Stop Solution	100 µl	100 µl	100 µl	
Incubate	5 min at room temperature (20-26°C/68-78.8°F)				
Agitate plate for 5 seconds and read OD at λ450nm (optionally λ 450/620 nm) within 30 minutes. Resulting color is stable for 30 minutes, at least.					



Annex B: Test Procedure





Annex C: Test Protocol

Assay/Test: _____ Incubation/ Inkub.: 1. _____ min Date/Datum: _____
Temperature/Temperatur: _____ °F _____ °C Signature/Unterschrift: _____
Name: _____ 2. _____ min
3. _____ min

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												