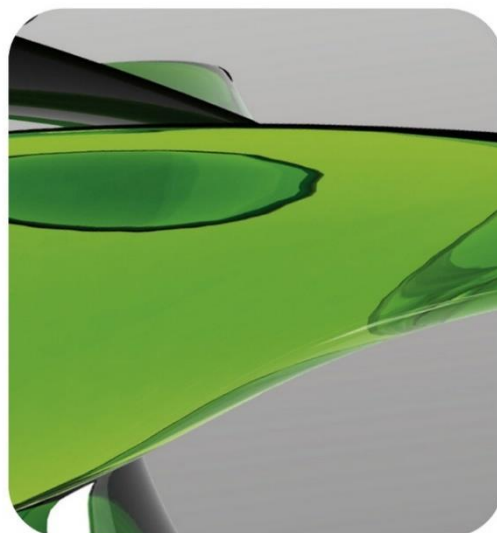




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AESKULISA®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA® U1-70

REF 7104US





Product Ref.	7104US
Product Desc.	U1-70
Manual Rev. No.	005: 2025-10-06

Instruction manual

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1 Intended Use

AESKULISA® U1-70 is a solid phase enzyme immunoassay employing recombinant human 70 kDa protein of the U1-snRNP complex for the semi-quantitative and qualitative detection of antibodies against the 70 kDa U1-RNP in human serum. The assay is an aid in the diagnosis of mixed connective tissue diseases (MCTD) 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

The U1-snRNP complex is a small nuclear ribonucleoprotein particle composed of uridine rich (thus U) small nuclear RNA and a set of proteins: the 70 kDa U1-specific ribonucleoprotein plus proteins A and C (all formerly summarized as RNPs) and the Sm (Smith) antigen which comprises eight proteins: B, B', D1, D2, D3, E, F, and G. Because of its protein components, the Sm and RNPs, the complex is often named RNP/Sm complex. The U1-snRNP complex is a part of the splicosomal complex, that facilitates the processing of pre-mRNA to mature mRNA in the nucleus.

Antibodies against the 70 kDa protein of the U1-snRNP complex belong to the heterogenous group of anti-nuclear antibodies (ANA), which are associated with various autoimmune diseases. They are directed against various proteins of the nucleus. Indirect immunofluorescence test (IFT) on eucaryotic cells like HeLa has been established method for the detection of ANAs. Single antibody specificities are distinguished by fluorescence patterns but more specific testing by ELISAs employing the target antigens are available too for a simple and reliable differentiation of ANAs.

Antibodies against the 70 kDa U1-specific protein are found in 95% of mixed connective tissue diseases (MCTD) but do also occur in systemic lupus erythematosus (SLE) with a prevalence of 40%. Isolated occurrence of anti-70 kDa antibodies is typical for the Sharp syndrome. Whilst antibodies against Sm are highly specific for systemic lupus erythematosus (SLE) and thus are included in diagnostic and classification criteria for SLE. Anti-Sm antibodies are found in 20-30% of patients with SLE.

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: yellow solutions)
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 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.



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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).
- 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 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

≤ 15 U/ml

Positive Results

> 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	0.044	3.2
3 U/ml	0.132	0.6
10 U/ml	0.300	2.1
30 U/ml	0.557	2.8
100 U/ml	1.224	1.9
300 U/ml	2.130	0.3

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	1.156/1.108	1.132	86.9
P 02	0.543/0.564	0.554	29.9

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 microplate is coated with **recombinant human 70 kDa U1-snRNP**.

No crossreactivities to other autoantigens have been found. Antibodies against 70 kDa protein of the U1 snRNP complex occur in different diseases which are shown in the table below:

Disease	prevalence
MCTD	95%
SLE	40%

A study with 20 Anti-U1-70 positive and 53 negative sera (from patients with various rheumatic disorders) and the **AESKULISA®** U1-70 is shown in the table below:

clinical data for U1-70	results from the AESKULISA U1-70		
		positive	negative
	positive	20	0
	negative	0	53

100% 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 Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%)
1	1 / 100	144.0	148.0	97.3
	1 / 200	73.5	74.0	99.3
	1 / 400	36.5	37.0	98.6
	1 / 800	18.0	18.5	97.3
2	1 / 100	54.3	55.0	98.7
	1 / 200	26.4	27.5	96.0
	1 / 400	12.9	13.8	93.5
	1 / 800	6.5	6.9	94.2

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			Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)	Sample No.	Mean (U/ml)	CV (%)
1	151.0	2.3	1	148.0	2.6
2	72.0	3.6	2	70.0	4.1
3	26.0	4.5	3	24.0	3.9

10.5 Calibration

The **AESKULISA®** U1-70 is calibrated against reference sera from the CDC (Centers for Disease Control and Prevention) Atlanta. The results are expressed in U/ml.



11 Literature

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12 Regulatory Symbols

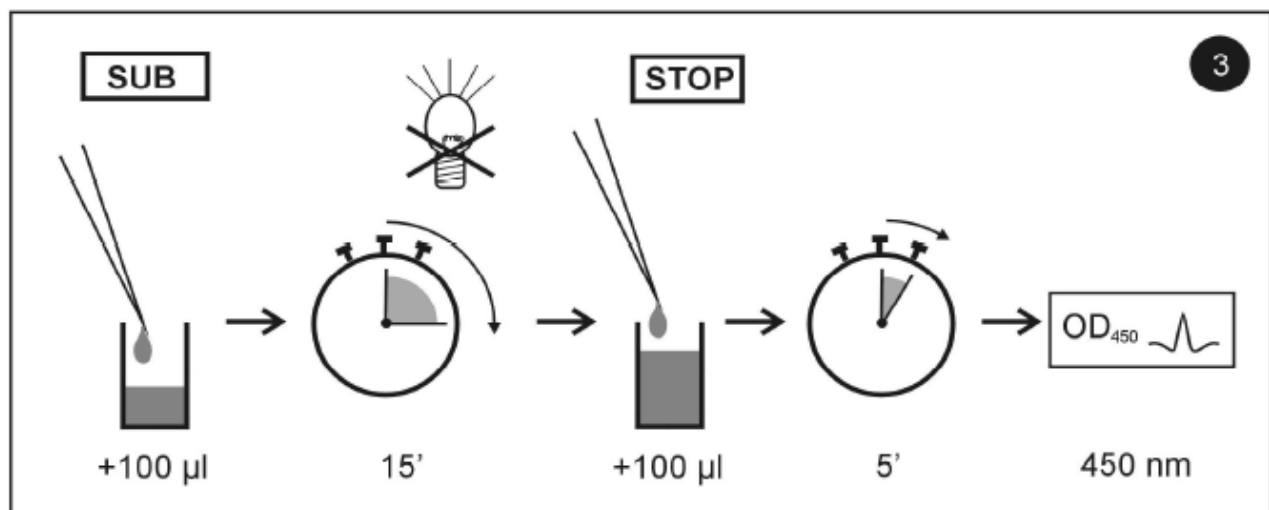
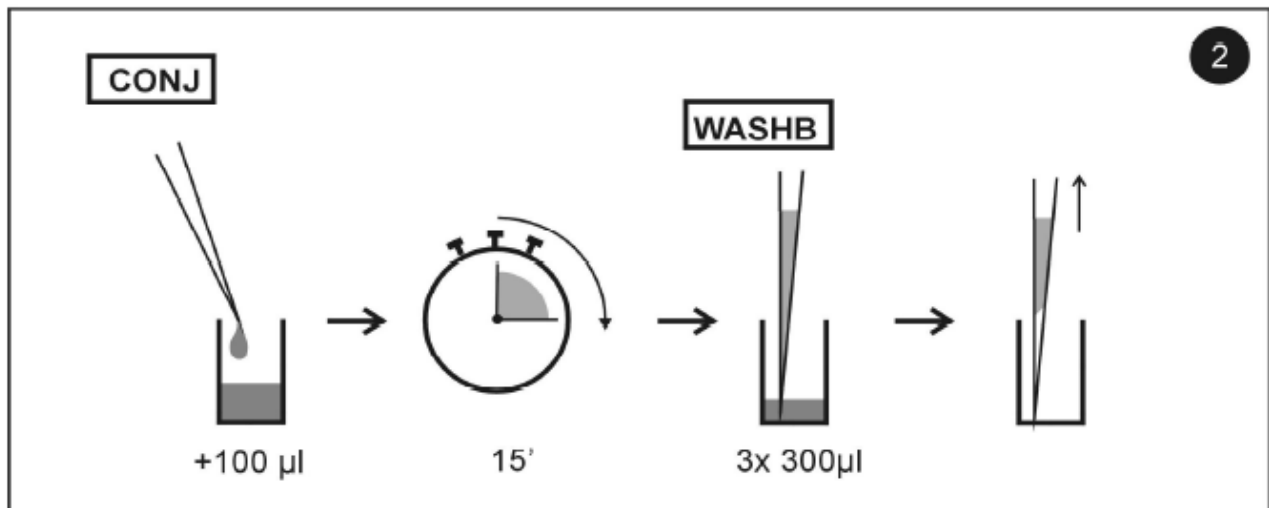
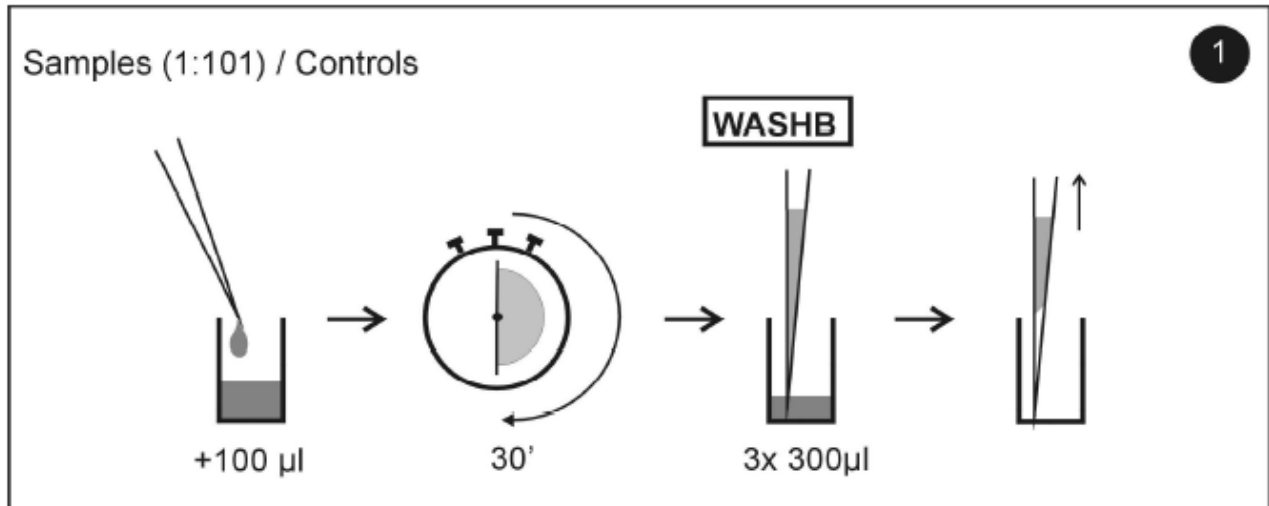
IVD	For in vitro diagnostic use
REF	Catalog number
LOT	Lot
UDI	Unique Device Identifier
	96 tests
	See instructions for use
	Use by
	Store at 2-8°C (35.6-46.4°F)
	Manufactured by
CON +	Positive Control
CON -	Negative Control
CO-CAL	Cut off Calibrator
CONJ	Conjugate
MP	Coated microtiter plate
WASHB 50x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

Annex

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.								

B: Test Procedure



C: Test Protocol

Assay/Test: _____

Temperature/Temperatur: _____ °F _____ °C

Name: _____

Incubation/ Inkub. : _____

1. _____ min

2. _____ min

3. _____ min

Date/Datum: _____

Signature/Unterschrift: _____

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

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