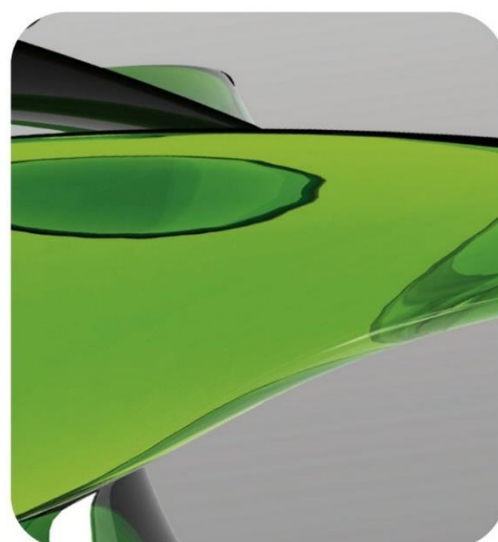




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THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA® ANA-HEp-2

REF 30-7115US





Product Ref.	30-7115US
Product Desc.	ANA-HEp-2
Manual Rev. No.	008: 2026-01-14

Instruction manual

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1 Intended Use

AESKULISA® ANA-HEp-2 is a solid phase enzyme immunoassay for the combined qualitative detection of IgG antibodies against HEp2 cells in human serum. Each well is coated with lysed HEp2 cells and specific antigens. The test collectively detects, in one well, total ANAs against double-stranded DNA (dsDNA), histones, SS-A (Ro), SS-B (La), Sm, snRNP/Sm, Scl-70, Jo-1 and centromeric antigens along with sera positive for HEp2 immunofluorescence test (IFT).

The assay is a tool in the diagnosis of certain systemic rheumatic diseases and should be used in conjunction with other serological tests and clinical findings.

2 Clinical Application and Principle of the Assay

Anti-nuclear antibodies (ANA) directed against a variety of nuclear and cytoplasmic antigens occur in high frequency in systemic rheumatic diseases and thus are an important tool for the differential diagnosis. For instance, SS-A (Ro) and SS-B (La) antibodies are associated with SLE and Sjögren's syndrome (SS), anti-dsDNA and anti-Sm antibodies with SLE, anti-histone antibodies with SLE and drug-induced lupus, anti-RNP antibodies with mixed connective tissue disease (MCTD) and SLE, anti-Scl-70 antibodies with scleroderma (progressive systemic sclerosis [PSS]), anti-Jo-1 antibodies with polymyositis and dermatomyositis and anti-centromere antibodies with CREST syndrome.

Indirect immunofluorescence test (IFT) on eucaryotic cells like HeLa and HEp2 has been the established method for the detection of ANAs. Although the IFT is a sensitive test, it is laborious when testing large numbers of patient samples and is subject to errors from human interpretation and from variability in fluorescent microscope. The ELISA test system is an excellent alternative to the IFT for screening patient's serum for the presence of ANAs of clinical significance. Single antibody specificities have to be determined by more specific testing using ELISAs employing the specific target antigens for a simple and reliable differentiation of ANAs.

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 < 0.1% (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide < 0.1% (preservative)

Ready to use:

Negative Control 1 vial, 1.5 ml (capped green: clear solution)
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Positive Control 1 vial, 1.5 ml (capped red: yellow solution)
Containing: Human serum (diluted), sodium azide < 0.1% (preservative)

Cut-off Control 1 vial, 1.5 ml (capped blue: yellow solution)
Containing: Human serum (diluted), sodium Azide < 0.1% (preservative)

Conjugate 1 vial, 15 ml IgG (capped blue: blue solution)
Containing: goat polyclonal 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 (600-690nm). 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 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.

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 FDA 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

For pipetting scheme see Annex A, for the test procedure see Annex B

- 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 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 TMB substrate into each well.
- Incubate for 30 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 Semiquantitative Interpretation

Read the optical density of the cut-off control and the patient samples. Compare patient ODs with the OD of the cut-off control. All samples which are higher than cut-off are considered positive. A positive result may indicate the presence of one or more autoantibodies. Confirmatory testing for individual autoantibody should be performed.

Negative: OD Patient < OD_{cut-off}

Positive: OD Patient > OD_{cut-off}

Calibrators	O.D. 450/620 nm	CV % (Variation)
Negative Control	0.081	2.6
Cut-off Control	0.52	1.8
Positive Control	1.259	0.7

Example of interpretation

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

Cut-off control	Patient sample	Interpretation
0.52 OD	0.25 OD	Negative
0.52 OD	0.52 OD	Borderline
0.52 OD	0.67 OD	Positive
0.52 OD	1.75 OD	Positive

Do not use this example for interpreting patients results!

We recommend to retest samples, that are borderline. 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.

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 OD:

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

Negative: Index Value < 1.0

Positive: Index Value > 1.0

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 room temperature (20-26°C/68-78.8°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 lysed HEp2 cells. No cross reactivities to other autoantigens have been found (tTG, PR3, TPO, TG, Gliadin). ANA are not specific for SLE but are found in a variety of rheumatic diseases. Detection of ANA is a very sensitive marker for an active SLE and is positive in > 99% of all cases.

57 characterized sera of patients suffering from various autoimmune (AI) diseases (SLE, MCTD, CREST and Sjögrens syndrome; see table below) obtained from major hospitals which were positive on IFA HEp-2 ANA ($\geq 1:160$) were tested on a predicate device and the **AESKULISA® ANA HEp-2**. Two sera which were negative in IFA were also found to be negative in the **AESKULISA® ANA HEp-2**. There was 100% agreement with the predicate device.

disease	# of tested sera
SLE	39
MCTD	3
CREST	4
Sjogrens S.	4
various AI diseases	7

		pred. device		
		+	-	Total
AESKULISA®	+	57	0	57
	-	0	2	2
	Total	57	2	

A control group (n=80) was found negative on the **AESKULISA® ANA HEp-2**.

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	4.10	4.200	97.6
	1 / 200	2.10	2.100	100.0
	1 / 400	1.00	1.050	95.2
	1 / 800	0.55	0.530	103.8
2	1 / 100	6.10	6.200	98.4
	1 / 200	3.00	3.100	96.8
	1 / 400	1.59	1.550	102.6
	1 / 800	0.79	0.775	102.0

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		
Sample No.	Mean (OD-Ratio)	CV (%)	Sample No.	Mean (OD-Ratio)	CV (%)
1	4.6	1.5	1	4.7	3.1
2	2.8	2.0	2	3.0	2.5
3	1.4	1.8	3	1.2	2.4

10.4 Calibration

The **AESKULISA® ANA HEp-2** is calibrated against reference sera from the CDC (center of disease control) Atlanta.

11 Literature

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Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology.
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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
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

A: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:

For **qualitative interpretation** use cut-off control

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC	P2										
B	NC	P2										
C	CC	P3										
D	CC	P3										
E	PC	...										
F	PC	...										
G	P1	...										
H	P1	...										

CC: Cut-off control

PC: positive control

NC: negative control

P1: patient 1

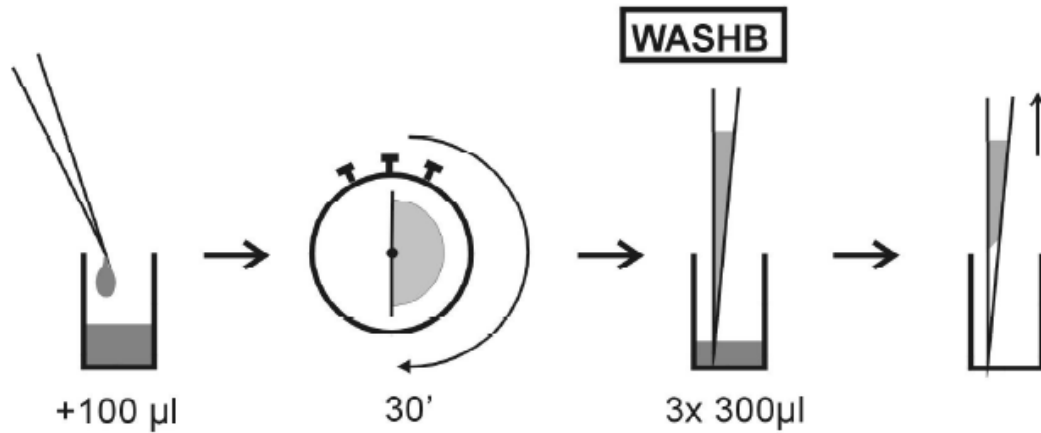
P2: patient 2

P3: patient 3

B: Test Procedure

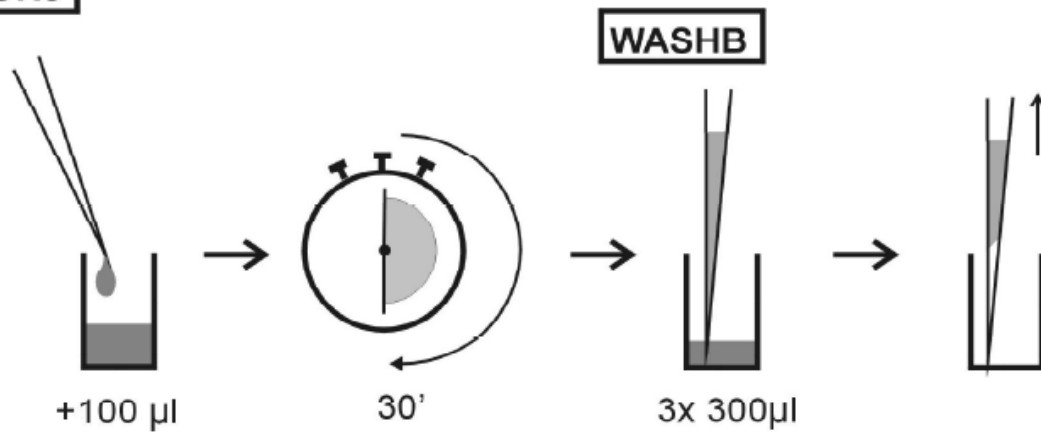
Samples (1:101) / Controls

1



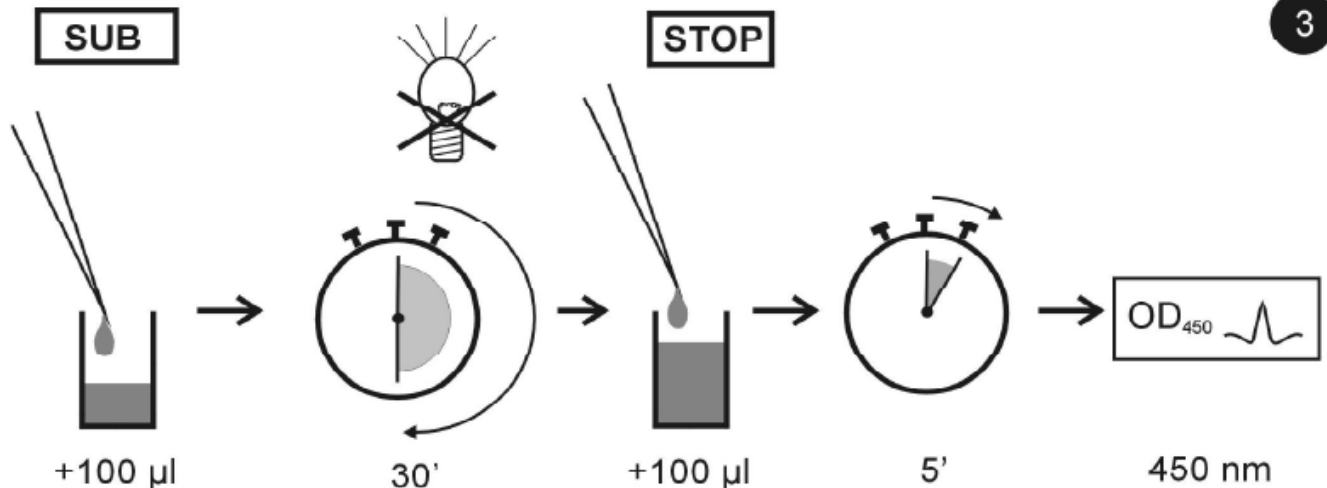
CONJ

2



SUB

3



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	Negative Control 0 U/ml											
B	Negative Control 0 U/ml											
C	Negative Control 0 U/ml											
D	Negative Control 0 U/ml											
E	Negative Control 0 U/ml											
F	Negative Control 0 U/ml											
G	Negative Control 0 U/ml											
H	Negative Control 0 U/ml											