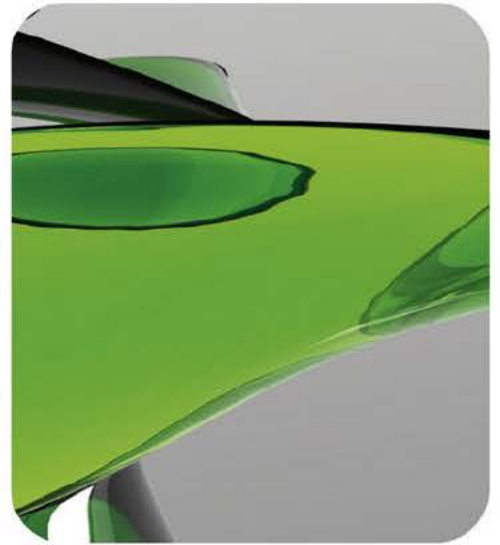




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**AESKULISA**<sup>®</sup>  
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# INSTRUCTION MANUAL

**AESKULISA ANA-HEp-2**

Ref 3115



و نديپاز



Product Ref.	3115
Product Desc.	ANA-HEp-2
Manual Rev. No.	004 : 2014-03-12

## 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. 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, PM-Scl, Jo-1 and centromeric antigens along with sera positive for HEp2 immunofluorescence test (IFT).

The assay is a tool in the diagnosis of systemic rheumatic diseases like systemic lupus erythematosus (SLE), mixed connective tissue diseases (MCTD), scleroderma, Sjögren's syndrome, polymyositis and dermatomyositis.

## 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 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

<b>TO BE RECONSTITUTED</b>				
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)
<b>READY TO USE</b>				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Blue	Blue	Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA),
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethy benzidine and hydrogen peroxide (TMB/H <sub>2</sub> O <sub>2</sub> )
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				
<b>MATERIALS REQUIRED, BUT NOT PROVIDED</b>				
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/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for at least 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.



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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 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 ( $\text{NaN}_3$ ) as a preservative.  $\text{NaN}_3$  may be toxic if ingested or adsorbed by skin or eyes.  $\text{NaN}_3$  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 ( $20\text{-}32^\circ\text{C}/68\text{-}89.6^\circ\text{F}$ ) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation: We recommend test performance at  $30^\circ\text{C}/86^\circ\text{F}$  for automated systems.**

Never expose components to higher temperature than  $37^\circ\text{C}/98.6^\circ\text{F}$ .

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

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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/35-46°F up to 48h, or frozen at -20°C/-4°F for longer periods

## 7 Assay Procedure

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### 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/35-46°F).



## 7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

	1	2	3	4...
A	NC	P2		
B	NC	P2		
C	CC	P3		
D	CC	P3		
E	PC	...		
F	PC	...		
G	P1	...		
H	P1	...		

PC: positive control

P1: patient 1


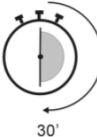
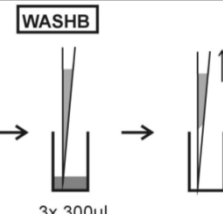
NC: negative control

P2: patient 2

CC: cut-off calibrator

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 qualitative interpretation results desired:
<b>CONTROLS &amp; SAMPLES</b>	
3.	 <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either: Cut-off Calibrator (CC) and 100 µl of each of the following:</p> <ul style="list-style-type: none"> <li>Negative control (NC) and Positive control (PC), and</li> <li>Patients diluted serum (P1, P2...)</li> </ul>
4.	 <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>
5.	 <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>





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### CONJUGATE

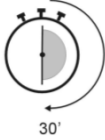
6.

**CONJ**



Pipette 100 µl conjugate into each well.

7.



Incubate for 30 minutes at 20-32°C/68-89.6°F.

8.

**WASHB**



Wash 3x with 300 µl washing buffer (diluted 1:50).

### SUBSTRATE

9.

**SUB**



Pipette 100 µl TMB substrate into each well.

10.

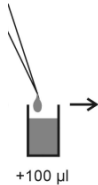


Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.

### STOP

11.

**STOP**



Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.

12.

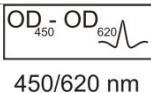


Incubate 5 minutes minimum.

13.

Agitate plate carefully for 5 sec.

14.



Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.



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## 8 Semiquantitative Interpretation

Read the optical density of the cut-off calibrator and the patient samples. Compare patient ODs with the OD of the cut-off calibrator. 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.

**Negative:** OD patient < 0.8 x OD cut-off  
**Equivocal:** 0.8 x OD cut-off ≤ OD patient ≤ 1.2 x OD cut-off  
**Positive:** OD patient > 1.2 x OD cut-off

Calibrators	O.D. 450/620 nm	CV % (Variation)
Negative Control	0.081	2.6
Cut-off Calibrator	0.350	1.8
Positive Control	1.259	0.7

### Example of interpretation.

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

Cut-off calibrator	Patient sample	OD Quotient	Interpretation
0.35 OD	0.25 OD	0.75	Negative
0.35 OD	0.40 OD	1.14	Equivocal
0.35 OD	0.56 OD	1.60	Positive
0.35 OD	1.75 OD	5.00	Positive

### 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 national 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 OD:

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

**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/35-46°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 crossreactivities 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 (≥1:160) were tested on a predicate device and the AESKULISA ANA-HEp-2. 2 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
Sjoegrens Syndrome	4
Various AI diseases	7

		Predicate device		
		Pos	Neg	Total
AESKULISA ANA-HEp-2	Pos	57	0	57
	Neg	0	2	2
		57	2	59

A controlgroup (n=80) were all 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 (Centers for Disease Control and Prevention) Atlanta

## 11 Literature

**Peter JB, Shoenfeld Y (1996).** Autoantibodies. Elsevier Sciences B.V., Amsterdam.




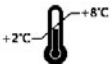

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**Mierau R, Genth E (1998).** Autoantikörper bei systemischem Lupus erythematodes und verwandten Erkrankungen In: Thomas L. (Hrsg.) Labor und Diagnose TH-Books, Frankfurt, 15. Auflage: 843-851.

**Schmolke M, Oppermann M, Helmke K, Guder WG (2000).** Antibody determination against ENA- a challenge for the routine laboratory Poster P59, 5 th Dresden Symposium on Autoantibodies.

**Tan EM, (1989).** Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv. Immunol 44: 93-151

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<b>IVD</b>	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγνωστικό
	- Para uso Diagnóstico in vitro	
<b>REF</b>	° Numero d'ordine	° Catalogue number
	° Référence Catalogue	° Numéro de catálogo
	° Bestellnummer	° Αριθμός παραγγελίας
<b>LOT</b>	° Número de catálogo	
	° Descrizione lotto	° Lot
	° Lot	° Lote
<b>CE</b>	° Chargen Bezeichnung	° Χαρακτηριστικός αριθμός παρτίδας
	° Lote	
	° Conformità europea	° EC Declaration of Conformity
	° Déclaration CE de Conformité	° Declaración CE de Conformidad
	° Europäische Konformität	° Εσοφπ αψη ζ ακθφλ ια
	° Déclaracão CE de Conformidade	
	° 96 determinazioni	° 96 tests
	° 96 tests	° 96 pruebas
	° 96 Bestimmungen	° 96 προζ δφρηζ κοί
	° 96 Testes	
	° Rispettare le istruzioni per l'uso	° See instructions for use
	° Voir les instructions d'utilisation	° Ver las instrucciones de uso
	° Gebrauchsanweisung beachten	° Λάβετε σπύε ηρη οδεγίεζ τρηζ εεζ
	° Ver as instruções de uso	
	° Da utilizarsi entro	° Use by
	° Utilise avant le	° Utilizar antes de
	° Verwendbar bis	° Χρηζ ε κέρρη
	° Utilizar antes de	
	° Conservare a 2-8°C	° Store at 2-8°C (35-46°F)
	° Conserver à 2-8°C	° Conservar a 2-8°C
	° Lagerung bei 2-8°C	° Φισζ ζεπηζ ηροζ 2-8°C
	° Conservar entre 2-8°C	
	° Prodotto da	° Manufactured by
<b>CO-CAL</b>	° Fabriqué par	° Fabricado por
	° Hergestellt von	° Καηηζ θεσδδρηηπό
	° Fabricado por	
	° Calibratore cut-off	° Cut off Calibrator
<b>CON+</b>	° Etalon Seuil	° Calibrador de cut-off
	° Grenzwert Kalibrator	° Ορηθόζ ορόζ Αληθραζ ηήρη ηαζκολόκεζεεζ
	° Calibrador de cut-off	
	° Controllo positivo	° Positive Control
<b>CON-</b>	° Contrôle Positif	° Control Positivo
	° Positiv Kontrolle	° Θεηθόζ ορόζ είε γτοσ
	° Controllo positivo	
	° Controllo negativo	° Negative Control
<b>CAL</b>	° Contrôle Négatif	° Control Negativo
	° Negativ Kontrolle	° Αρηε ηθόζ ορόζ είε γτοσ
	° Controllo negativo	
	° Calibratore	° Calibrator
<b>RC</b>	° Etalon	° Calibrador
	° Kalibrator	° Αληθραζ ηήρη ηαζκολόκεζεεζ
	° Calibrador	
	° Recupero	° Recovery
<b>CONJ</b>	° Corrélation	° Recuperado
	° Wiederfindung	° Αλάθηε ζε
	° Recuperação	
	° Coniugato	° Conjugate
<b>MP</b>	° Conjugé	° Conjugado
	° Konjugat	° Σύδωγκα
	° Conjugado	
	° Micropiastro rivestita	° Coated microtiter plate
<b>WASHB 50x</b>	° Microplaque sensibilisée	° Microplaca sensibilizada
	° Beschichtete Mikrotiterplatte	° Επηθασ κ κέλε κίθροηιάθα
	° Microplaca revestida	
	° Tampone di lavaggio	° Wash buffer
<b>SUB</b>	° Tampon de Lavage	° Solución de lavado
	° Waschpuffer	° Ραζ κίε ηθό δίηη σκα ηύ ζεεζ
	° Solução de lavagem	
	° Tampone substrato	° Substrate buffer
<b>STOP</b>	° Substrat	° Tampón sustrato
	° Substratpuffer	° Ραζ κίε ηθό δίηη σκα σποζ ηρώκαηρη
	° Substrato	
	° Reagente bloccante	° Stop solution
<b>SB 5x</b>	° Solution d'Arrêt	° Solución de parada
	° Stopreagenz	° Αληθραζ ηήρη δίηηοηήζ αληθραζ εεζ
	° Solução de paragem	
	° Tampone campione	° Sample buffer
	° Tampon Echantillons	° Tampón Muestras
	° Probenpuffer	° Ραζ κίε ηθό δίηη σκα δεηγηάηρη
	° Diluente de amostra	