# AESKULISA RA/CP-Detect

Ref 3165













Product Ref.	3165
Product Desc.	RA/CP-Detect
Manual Rev. No.	003 : 2013-10-10

## **Instruction Manual**

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#### 1 Intended Use

**AESKULISA RA/CP-Detect** is a solid phase enzyme immunoassay for the quantitative and qualitative detection of IgG antibodies to specific synthetic citrullinated peptides of human immunoglobulin G (IgG) in human serum.

The assay is an aid in the diagnosis of rheumatoid arthritis (RA).

## 2 Clinical Application and Principle of the Assay

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting approximately 1% of the population worldwide. It is a chronic, systemic, inflammatory disease that predominantly affects the synovial membranes of multiple joints in the body.

Up to now, rheumatoid factors (RF) have been the main serological markers for this disease. Rheumatoid factors, first described in 1940 as antibodies reacting with gamma globulins, are autoantibodies directed against the C-terminal part of the constant region of the IgG heavy chain, the IgG Fc.

Although named after the disease they were initially associated with, RFs are found both in the healthy population and several diseases. The diseases commonly associated with high RF concentrations are rheumatoid arthritis (50-90%) and Sjögren's syndrome (75-95%). They are also found in systemic lupus erythematosus (SLE; 15-35%), systemic sclerosis (20-30%) polymyositis / dermatomyositis (5-10%), cryoglobulinemia (40-100%) and mixed connective tissue diseases (MCTD; 50-60%). Therefore, despite being a sensitive marker for RA, rheumatoid factor lack specificity for the disease.

It has long been known that anti-perinuclear autoantibodies, also called anti-keratin antibodies, are found in patients with RA. Recently it has been discovered that these autoantibodies target an epitope containing the deimidated form of arginine called citrulline. Antibodies against synthetic cyclic citrullinated peptides (CCP) have been described as being highly specific markers for RA with sensitivities comparable to RF.

AESKU.DIAGNOSTICS has developed a novel ELISA test, the AESKULISA RA/CP-Detect, which detects sera from patients with rheumatoid arthritis with comparable sensitivities and specificities as CCP ELISA tests. By employing specific synthetic peptides from human immunoglobulin G, the AESKULISA RA/CP-Detect represents a highly specific serological test for the diagnosis of RA and is an adequate and cost-effective alternative to the CCP ELISA tests. Studies have shown a sensitivity of 68% and a specificity of 92% in a control group of overlapping rheumatic diseases.

## 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.



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## 3 Kit Contents

		TO BE I	RECONSTITU	JTED
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)
		RE	ADY TO USE	
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)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. 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 tetramethylbenzidine 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.

<sup>\*</sup> 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/35-46°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 (NaN3) as a preservative. NaN3 may be toxic if ingested or adsorbed by skin or eyes. NaN3 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.



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## 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/35-46°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/35-46°F).



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## 7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

## For QUANTITATIVE interpretation

	1	2	3	4
Α	Cal A	Cal E	P1	
В	Cal A	Cal E	P1	
С	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC		
Н	Cal D	NC		

#### For QUALITATIVE interpretation

	1	2	3	4
Α	NC	P2		
В	NC	P2		
С	CC	P3		
D	CC	P3		
E	PC			
F	PC			
G	P1			
Н	P1			

CalA: calibrator A
CalB: calibrator B
CalC: calibrator C

Description

CalD: calibrator D
CalE: calibrator E
CalF: calibrator F

PC: positive control P1: patient 1

NC: negative control P2: patient 2

CC: cut-off calibrator P3: patient 3

7.3 Test Steps

Step

## 7.5 Test Steps

- 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 either:

- a. Calibrators (CAL.A to CAL.F) for QUANTITATIVE or
- b. Cut-off Calibrator (CC) for QUALITATIVE interp.

and 100 µl of each of the following:

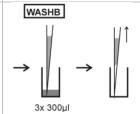
- Negative control (NC) and Positive control (PC), and
  - Patients diluted serum (P1, P2...)

4.



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

5.



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



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	CONJUGATE				
6.	+100 µl	Pipette 100 μl conjugate into each well.			
7.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.			
8.	<b>WASHB</b> →	Wash 3x with 300 μl washing buffer (diluted 1:50).			
		SUBSTRATE			
9.	SUB +100 μl	Pipette 100 μl TMB substrate into each well.			
10.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.			
		STOP			
11.	STOP  → +100 μI	Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.			
12.	5'	Incubate 5 minutes minimum.			
13.		Agitate plate carefully for 5 sec.			
14.	OD <sub>450</sub> OD <sub>620</sub> 450/620 nm	Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.			



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## 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	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

#### Example of a standard curve

Do NOT use this example for interpreting patient's result

Calibrators IgG	OD 450/620 nm	CV % (Variation)
0 U/ml	0.080	2.9
3 U/ml	0.166	3.0
10 U/ml	0.297	1.9
30 U/ml	0.619	2.6
100 U/ml	1.358	2.2
300 U/ml	2.250	0.2

#### Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.968/ 1.016	0.993	62.1
P 02	0.634/ 0.654	0.642	31.8

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min. 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 **qualitative interpretation** read the optical density of the cut-off calibrator and the patient samples. Compare patient's OD with the OD of the cut-off calibrator. 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.

Negative: OD patient < 0.8 x OD cut-off

Equivocal:  $0.8 \times OD \text{ cut-off} \leq OD \text{ patient } \leq 1.2 \times OD \text{ cut-off}$ 

Positive: OD patient > 1.2 x OD cut-off



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#### 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

Calibration range: 0-300 U/ml

Analytical sensitivity: 1.0 U/ml

Storage: at 2-8°C/35-46°F use original vials only.

Number of determinations: 96 tests

## 10 Performance Data

## 10.1 Analytical sensitivity

Testing sample buffer 30 times on AESKULISA RA/CP-Detect gave an analytical sensivity of 1.0 U/ml.

## 10.2 Specificity and sensitivity

The microplate is coated with specific synthetic citrullinated peptides of human immunoglobulin G (IgG). No crossreactivities to other autoantigens have been found. For rheumatoid arthritis the RA/CP-Detect ELISA shows a sensitivity of 68% and a specificity of 92% in a control group of overlapping rheumatic diseases.

## 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	Dilution	measured	expected	Recovery(%)
No.	Factor	concentration	concentration	
		(U/ml)	(U/ml)	
1	1 / 100	51.1	53.4	95.7
	1 / 200	25.2	26.7	94.4
	1 / 400	12.4	13.4	92.5
	1 / 800	6.3	6.7	94.0
2	1 / 100	135.1	138.0	97.9
	1 / 200	74.0	69.0	107.2
	1 / 400	32.1	34.5	93.0
	1 / 800	16.1	17.3	93.0



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#### 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.

Ir	ntra-assay	
Sample No.	Mean (U/ml)	CV (%)
1	15.2	0.4
2	43.4	4.5
3	288.8	8.9

Ir	iter-assay	
Sample No.	Mean (U/ml)	CV (%)
1	18.3	1.0
2	52.1	4.6
3	322.7	8.2

#### 10.5 Calibration

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

#### 11 Literature

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Corrélation   Recuperado   Wiederfindung   Recuperado   Wiederfindung   Recuperacão   Recuperação   Conjugate   Conjugate   Conjugate   Conjugado   Microplaque sensibilisée   Microplaça sensibilisée   Microplaça sensibilisée   Microplaça sensibilisée   Rimadupµévη μικροπλάκα   Coated microtiter plate   Microplaça sensibilisée   Conjugado   Coated microtiter plate   Conjugado   Coated microtiter plate   Conjugado   Coated microtiter plate   Conjugado   Coated microtiter plate   Microplaça sensibilisada   Coated microtiter plate   Conjugado   Coated microtiter plate   Coated microtiter plate   Coated microtiter plate   Coated mic	CON -	"Controllo positivo "Contrôle Positif "Positiv Kontrolle "Controlo positivo "Controllo negativo "Contrôle Négatif "Negativ Kontrolle "Controlo negativo "Calibratore "Etalon "Kalibrator	" Control Positivo " Θετικός ορός ελέγχου " Negative Control " Control Negativo " Αρνητικός ορός ελέγχου " Calibrator " Calibrador
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CONJ  Conjugé Konjugat  Conjugado  Microplastra rivestita  Microplaque sensibilisée Beschichtete Mikrotiterplatte Microplaca revestida  Microplaca revestida  Tampone di lavaggio  Tampone de Lavage Solucão de lavagem  Tampone substrato  Substrat  Substrate  Substrate  Reagente bloccante  Substrate  Reagente bloccante  Subcia de paragem  Solucão de paragem  Reagente  Subcia de paragem  Tampone campione  Tampon Echantillons  Probenpuffer  Probenpuffer  Probenpuffer  Puθμιστικό διάλυμα σειγμάτων		" Controllo positivo " Contrôle Positif " Positiv Kontrolle " Controlo positivo " Controllo negativo " Contrôle Négatif " Negativ Kontrolle " Controlo negativo " Calibratore " Etalon " Kalibrator " Calibrador " Recupero " Corrélation " Wiederfindung	" Control Positivo " Θετικός ορός ελέγχου " Negative Control " Control Negativo " Αρνητικός ορός ελέγχου " Calibrator " Calibrador " Αντιδραστήριο βαθμονόμησης " Recovery " Recuperado
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SB       5x         "Tampone campione       "Sample buffer         "Tampon Echantillons       "Tampón Muestras         "Probenpuffer       "Ρυθμιστικό διάλυμα δειγμάτων	RC CONJ MP WASHB 50x SUB	"Controllo positivo "Contrôle Positif "Positiv Kontrolle "Controlo positivo "Controllo negativo "Controllo negativo "Controllo negativo "Controlo negativo "Calibratore "Etalon "Kalibrator "Calibrador "Recupero "Corrélation "Wiederfindung "Recuperacão "Conjugato "Conjugato "Conjugato "Micropiastra rivestita "Microplaque sensibilisée "Beschichtete Mikrotiterplatte "Microplaca revestida "Tampone di lavaggio "Tampon de Lavage "Waschpuffer "Solucão de lavagem "Tampone substrato "Substrat "Substrato "Reagente bloccante "Solution d'Arrêt	" Control Positivo " Θετικός ορός ελέγχου " Negative Control " Control Negativo " Αρνητικός ορός ελέγχου " Calibrator " Calibrador " Αντιδραστήριο βαθμονόμησης " Recovery " Recuperado " Ανάκτηση " Conjugate " Conjugate " Conjugate " Conjugado " Σύζευγμα " " Coated microtiter plate " Μίcroplaca sensibilizada " Επικαλυμμένη μικροπλάκα " Wash buffer " Solución de lavado " Ρυθμιστικό διάλυμα πλύσης " Substrate buffer " Τampón sustrato " Ρυθμιστικό διάλυμα υποστρώματος " Stop solution " Solución de parada
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	RC CONJ MP WASHB 50x SUB STOP	Controllo positivo Contrôle Positif Positiv Kontrolle Controlo positivo Controllo negativo Contrôle Négatif Negativ Kontrolle Controlo negativo Calibratore Etalon Kalibrator Calibrador Recupero Corrélation Wiederfindung Recuperacão Conjugato Conjugat Konjugat Conjugat Micropiastra rivestita Microplacu e sensibilisée Beschichtete Mikrotiterplatte Microplaca revestida Tampone de lavage Waschpuffer Solucão de lavagem Tampone substrato Substrat Substrat Reagente bloccante Solution d'Arrêt Stopreagenz Solucão de paragem Tampone campione Tampone campione Tampone Echantillons Probenpuffer	" Control Positivo " Θετικός ορός ελέγχου " Negative Control " Control Negativo " Αρνητικός ορός ελέγχου " Calibrator " Calibrator " Αντιδραστήριο βαθμονόμησης " Recovery " Recuperado " Ανάκτηση " " Conjugate " Conjugate " Conjugate " Conjugato " Σύζευγμα " " Coated microtiter plate " Μίcroplaca sensibilizada " Επικαλυμμένη μικροπλάκα " Wash buffer " Solución de lavado " Ρυθμιστικό διάλυμα πλύσης " Substrate buffer " Ταπρόη sustrato " Ρυθμιστικό διάλυμα υποστρώματος " Stop solution " Solución de parada " Αντιδραστήριο διακοπής αντίδρασης " Sample buffer " Ταπρόη Muestras