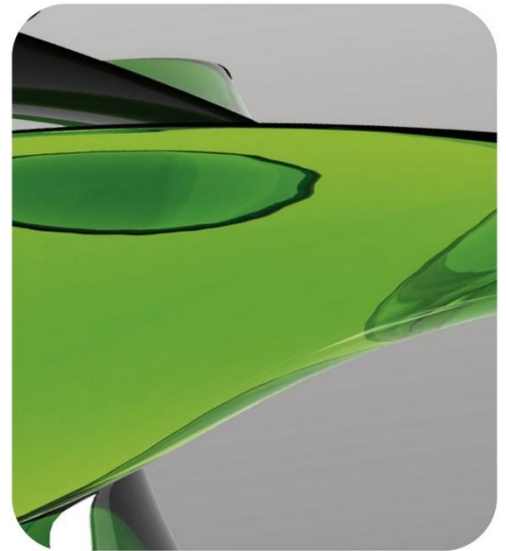




AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS



AESKULISA[®]

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA CCP

Ref 3166





Product Ref.	3166
Product Desc.	CCP
Manual Rev. No.	004 : 2017-08-28

Instruction Manual

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1 Intended Use

AESKULISA CCP is a solid phase enzyme immunoassay for the quantitative and qualitative detection of IgG antibodies to specific cyclic citrullinated peptides.

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 deiminated 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 CCP, which detects sera from patients with rheumatoid arthritis with comparable sensitivities and specificities as CCP ELISA tests. By employing specific cyclic citrullinated peptides, the AESKULISA CCP 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 75% and a specificity of 99,2% 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.

3 Kit Contents

TO BE RECONSTITUTED				
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)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Calibrator material (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. Calibrator material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Blue	Blue	Containing: Immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H ₂ O ₂)
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				
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 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.

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 (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 biological source material used for some reagents of this kit 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 these 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.

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. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4)

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).

7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For <i>QUANTITATIVE</i> interpretation					For <i>QUALITATIVE</i> interpretation				
	1	2	3	4...		1	2	3	4...
A	Cal A	P1			A	NC	...		
B	Cal B	P2			B	CC	...		
C	Cal C	P3			C	CC	...		
D	Cal D	...			D	PC			
E	Cal E	...			E	P1			
F	Cal F	...			F	P2			
G	PC				G	P3			
H	NC				H	...			

CalA: calibrator A

CalD: calibrator D

PC: positive control

P1: patient 1

CalB: calibrator B

CalE: calibrator E

NC: negative control

P2: patient 2

CalC: calibrator C


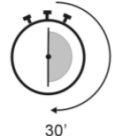
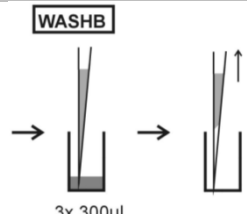
CalF: calibrator F

CC: cut-off calibrator

P3: patient 3

It is good laboratory practice to run calibrators, controls and samples in duplicate.

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 quantitative/ qualitative interpretation results desired:
CONTROLS & SAMPLES	
3.	 <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:</p> <ol style="list-style-type: none"> Calibrators (CAL.A to CAL.F) for <i>QUANTITATIVE</i> or Cut-off Calibrator (CC) for <i>QUALITATIVE</i> interp. <p>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> Negative control (NC) and Positive control (PC), and Patients diluted serum (P1, P2...)
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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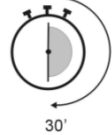
CONJUGATE

6. **CONJ**



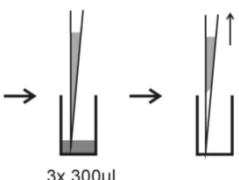
Pipette 100 µl conjugate into each well.

7.



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

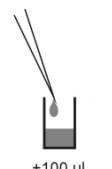
8. **WASHB**



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

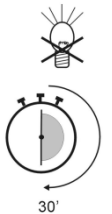
SUBSTRATE

9. **SUB**



Pipette 100 µl TMB substrate into each well.

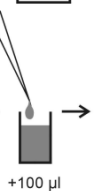
10.



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.

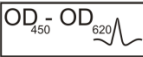


Incubate 5 minutes minimum.

13. **AGITATE**

Agitate plate carefully for 5 sec.

14. **OD₄₅₀ - OD₆₂₀**



Read absorbance at 450 nm (recommended 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	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	0.038	2.8
3 U/ml	0.165	2.9
10 U/ml	0.334	4.5
30 U/ml	0.637	3.2
100 U/ml	1.219	3.8
300 U/ml	2.025	1.0

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.896/ 0.815	0.842	55.2
P 02	1.813/ 1.805	1.809	230

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 x OD cut-off	≤	OD patient ≤ 1.2 x OD 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:	0.33 U/ml
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Normal Range

Sera of healthy donors have been investigated on AESKULISA CCP and resulted in the following distribution:

Number of Samples	negative	borderline	positive
295	287 (97.3 %)	3 (1 %)	5 (1.7%)

We also recommend that each laboratory should establish its own normal range.

10.2 Precision

Precision of test results obtained with AESKULISA CCP, REF 3166 were assessed by the determination of the intra- and inter assay precision as well as the lot-to-lot variance by the analysis of multiple samples of different antibody activities.

Sample ID	Intra Assay Precision		Inter Assay Precision		LOT to LOT Precision	
	Mean (U/ml)	CV	Mean (U/ml)	CV	Mean (U/ml)	CV
Sample 1	4.3	12.0%	4.3	15.9%	3.7	20.6%
Sample 2	14.2	10.5%	14.2	15.5%	14.2	15.8%
Sample 3	59.6	6.8%	59.6	7.4%	57.3	10.3%
Sample 4	91.1	8.7%	91.1	12.1%	92.6	9.3%
Sample 5	192.1	5.7%	192.1	6.9%	191.2	7.8%
Sample 6	319.6	9.9%	319.6	10.6%	308.2	9.7%

10.3 Sensitivity and Specificity

Analytical sensitivity

The analytical sensitivity has been assessed by multiple analysis of sample buffer and low positive samples and calculating the limit of detection.

For AESKULISA CCP, REF 3166 a **LoD of 0.33 U/ml** has been determined.



10.4 Linearity

Three sera covering the whole test range were diluted serially with a negative serum sample. Measured and expected values of the distinct dilutions were used to calculate a linear regression. According to results of linearity testing a measurable range of 3 - 300 U/ml was determined for AESKULISA CCP.

10.5 Calibration

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

11 Disposal

Please observe the relevant statutory requirements!

12 Literature

Witte T, Hartung K, Sachse C, Matthias T, Fricke M, Kalden JR, Lakomek HJ, Peter HH, Schmidt RE (2000). Rheumatoid factors in systemic lupus erythematosus: Association with clinical and laboratory parameters. SLE study group. *Rheumatol Int* 19: 107-111.






Smith JB, Haynes MK (2002). Rheumatoid arthritis - a molecular understanding. *Ann Intern Med* 136: 908 - 922.

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Schellekens GA, de Jong BAW, van den Hoogen FHJ, van de Putte LBA, van Venrooij WJ (1998). Citrulline is an essential constituent of antigenic determinants recognized by rheumatoid arthritis-specific autoantibodies. *J Clin Invest* 101: 273-281.

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CLSI Guideline GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

IVD	- Diagnosi in vitro - Pour diagnostic in vitro - In Vitro Diagnostikum - Para uso Diagnóstico in vitro	- For in vitro diagnostic use - Para uso diagnóstico in vitro - In Vitro Διαγνωστικό μέσο
REF	" Numero d'ordine " Référence Catalogue " Bestellnummer " Número de catálogo	" Catalogue number " Numéro de catálogo " Αριθμός παραγγελίας
LOT	" Descrizione lotto " Lot " Chargen Bezeichnung " Lote	" Lot " Lote " Χαρακτηρισμός παρτίδας
CE	" Conformità europea " Déclaration CE de Conformité " Europäische Konformität " Declaração CE de Conformidade	" EC Declaration of Conformity " Declaración CE de Conformidad " Ευρωπαϊκή συμφωνία
	" 96 determinazioni " 96 tests " 96 Bestimmungen " 96 Testes	" 96 tests " 96 pruebas " 96 προσδιορισμοί
	" Rispettare le istruzioni per l'uso " Voir les instructions d'utilisation " Gebrauchsanweisung beachten " Ver as instruções de uso	" See instructions for use " Ver las instrucciones de uso " Λάβετε υπόψη τις οδηγίες χρήσης
	" Da utilizzarsi entro " Utilise avant le " Verwendbar bis " Utilizar antes de	" Use by " Utilizar antes de " Χρήση μέχρι
	" Conservare a 2-8°C " Conserver à 2-8°C " Lagerung bei 2-8°C " Conservar entre 2-8°C	" Store at 2-8°C (35-46°F) " Conservar a 2-8°C " Φυλάσσεται στους 2-8°C
	" Prodotto da " Fabriqué par " Hergestellt von " Fabricado por	" Manufactured by " Fabricado por " Κατασκευάζεται από
CO-CAL	" Calibratore cut-off " Etalon Seuil " Grenzwert Kalibrator " Calibrador de cut-off	" Cut off Calibrator " Calibrador de cut-off " Οριακός ορός Αντιδραστήριο βαθμονόμησης
CON+	" Controllo positivo " Contrôle Positif " Positiv Kontrolle " Controllo positivo	" Positive Control " Control Positivo " Θετικός ορός ελέγχου
CON-	" Controllo negativo " Contrôle Négatif " Negativ Kontrolle " Controllo negativo	" Negative Control " Control Negativo " Αρνητικός ορός ελέγχου
CAL	" Calibratore " Etalon " Kalibrator " Calibrador	" Calibrator " Calibrador " Αντιδραστήριο βαθμονόμησης
RC	" Recupero " Corrélation " Wiederfindung " Recuperação	" Recovery " Recuperado " Ανάκτηση
CONJ	" Coniugato " Conjugé " Konjugat " Conjugado	" Conjugate " Conjugado " Σύζευγμα
MP	" Micropiastra rivestita " Microplaque sensibilisée " Beschichtete Mikrotiterplatte " Microplaca revestida	" Coated microtiter plate " Microplaca sensibilizada " Επικαλυμμένη μικροπλάκα
WASHB 50x	" Tampone di lavaggio " Tampon de Lavage " Waschpuffer " Solução de lavagem	" Wash buffer " Solución de lavado " Ρυθμιστικό διάλυμα πλύσης
SUB	" Tampone substrato " Substrat " Substratpuffer " Substrato	" Substrate buffer " Tampón sustrato " Ρυθμιστικό διάλυμα υποστρώματος
STOP	" Reagente bloccante " Solution d'Arrêt " Stopreagenz " Solução de paragem	" Stop solution " Solución de parada " Αντιδραστήριο διακοπής αντίδρασης
SB 5x	" Tampone campione " Tampon Echantillons " Probenpuffer " Diluente de amostra	" Sample buffer " Tampón Muestras " Ρυθμιστικό διάλυμα δειγμάτων