AESKULISA ASCA-A

Ref 3507













Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

Instruction Manual

Table of Contents

1	Intended Use	1
2	Clinical Application and Principle of the Assay	1
3	Kit Contents	2
4	Storage and Shelf Life	2
5	Precautions of Use	3
6	Sample Collection, Handling and Storage	4
7	Assay Procedure	4
8	Quantitative and Qualitative Interpretation	7
9	Technical Data	8
10	Performance Data	8
11	Literature	10



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Manual Rev. No.	003 : 2013-10-10

1 Intended Use

AESKULISA ASCA-A is a solid phase enzyme immunoassay (ELISA) employing highly purified mannan for the quantitative and qualitative detection of IgA anti-Saccharomyces cerevisiae antibodies (ASCA) in human serum. ASCA recognize specifically mannan, a component of the outer cell wall of yeast. The assay is highly specific and sensitive for Crohn's disease.

2 Clinical Application and Principle of the Assay

Crohn's disease is one of the two major Inflammatory Bowel Diseases (IBD). IBD is an umbrella term, that covers both primary disorders causing inflammation or ulceration in the small and large intestine, Crohn's disease and ulcerative colitis. Crohn's disease affects both, the small bowel and the colon, unlike ulcerative colitis which is restricted to the colon only. The etiology is not revealed yet, although a genetic and infectious background for the disease is under discussion. Colonoscopy and ileoscopy are the established tools of diagnosis, no serology was available so far. Though Crohn's disease and ulcerative colitis share several symptoms the course of the diseases, its complications and its management are different, especially when it comes down to surgery. Consequently the differental diagnosis of both diseases is crucial prior to treatment. Aggravating, about 5-10% of the patients can not be distinguished clearly by existing available diagnostic methodologies and are referred to as indeterminate colitis.

ASCA have been found to be specific markers for Crohn's disease. They have been reported for these patients with a frequency of 68%. The identification of the target antigen mannan, a mannose-rich carbohydrate antigen of the outer cell wall of yeast, enabled the detection of ASCA by enzyme immunoassay.

Being the first available highly specific serological marker, ASCA may become an important tool for the difficult task of diagnosing IBD. Moreover its high positive predictive value offers the only possibility of a convenient and reliable screening and monitoring of risk groups.

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.



Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

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)
		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, IgA	1 x 15ml	Red	Red	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 ₂ O ₂)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate * Color increasing with concentration	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.).

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.



Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

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.



Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

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).



Product Ref.		3507
Product Desc.		ASCA-A
	Manual Rev. No.	003 : 2013-10-10

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	СС	P3		
D	СС	P3		
E	PC			
F	PC			
G	P1			
Н	P1			

CalA: calibrator A CalD: calibrator D Pole CalB: calibrator B CalE: calibrator E No CalC: calibrator C CalF: calibrator F CalC: calibrator C CalF: calficr C Ca

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 fr	om step 7.1 above have been carried out prior to pipetting.
2.	Use the following step results desired:	s in accordance with quantitative/ qualitative interpretation
		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 orb. Cut-off Calibrator (CC) for QUALITATIVE interp.
		and 100 µl of each of the following:
	+100 µl	 Negative control (NC) and Positive control (PC), and Patients diluted serum (P1, P2)
4.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	WASHB →	Wash 3x with 300 μl washing buffer (diluted 1:50).



Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

	CONJUGATE				
6.	+100 hl	Pipette 100 μl conjugate into each well.			
7.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.			
8.	WASHB → 3x 300µI	Wash 3x with 300 μl washing buffer (diluted 1:50).			
		SUBSTRATE			
9.	*100 µI	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.	+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 ₅₀ OD ₆₂₀ 450/620 nm	Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.			



Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

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 IgA	OD 450/620 nm	CV % (Variation)
0 U/ml	0.032	2.8
3 U/ml	0.152	2.6
10 U/ml	0.281	1.2
30 U/ml	0.646	2.4
100 U/ml	1.214	1.7
300 U/ml	2.104	1.6

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.904/0.937	0.921	58.3
P 02	0.564/0.551	0.558	25.9

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



Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

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 ASCA-A gave an analytical sensivity of 1.0 U/ml.

10.2 Clinical Evaluation

The microplates are coated with purified mannan from S. cerevisiae. 264 sera suffering from Crohns disease, Ulcerative Colitis and several other diseases have been tested on the AESKULISA ASCA-A and another ASCA test.

Disease	# Tested	# positive AESKU	% positive AESKU
Crohns Disease	100	59	59.0
Ulcerative Colitis	55	9	16.4
Healthy donors	50	0	0.0
Celiac Disease	30	2	6.7
Systemic Lupus Erythematosus	10	2	20.0
Wegeners Granulomatosis	2	0	0.0
Sjörgrens Syndrome	4	2	50.0
Reactive Arthritis	11	1	9.1
Mixed Connective Tissue disease	1	0	0.0
Chronic Arthritis	1	0	0.0



	Product Ref.	3507
Product Desc.		ASCA-A
Manual Rev. No.		003 : 2013-10-10

10.3 Method Comparison

		other ASCA A assay		
		Pos	Neg	Total
AESKULISA	Pos	55	20	75
ASCA-A	Neg	19	170	189
	Total	74	190	264

pos. agreement: 74.3 % (55/74)
neg. agreement: 89.5 % (170/190
total agreement: 85.2 % (225/264)

Be advised that the agreement refers to the comparison of the AESKULISA ASCA-A results to that of another ASCA A kit. There was no attempt to correlate the assay results with the disease presence or absence. No judgement can be made on the comparisons accuracy to predict disease.

10.4 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	(U/ml)	(U/ml)	(%)
1	1 / 100	124.4	125.0	99.5
	1 / 200	63.6	62.5	101.7
	1 / 400	33.4	31.3	106.9
	1 / 800	16.0	15.6	102.4
2	1 / 100	289.5	295.0	98.1
	1 / 200	154.7	147.5	104.9
	1 / 400	70.6	73.8	95.8
	1 / 800	33.7	36.9	91.3

10.5 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. (n=18). The accepted range for CV is 10%. (n=24 / 18)

Intra-assay				
Sample No. Mean (U/ml) CV (%)				
1	18.2	5.6		
2	52.5	5.1		
3	112.7	2.9		

Inter-assay			
Sample No.	Mean (U/ml)	CV (%)	
1	23.1	4.8	
2	48.5	4.7	
3	115.6	3.7	

10.6 Calibration

Due to the lack of international reference calibration AESKULISA ASCA-A is calibrated in arbitrary units (U/ml).



Product Ref.	3507
Product Desc.	ASCA-A
Manual Rev. No.	003 : 2013-10-10

11 Literature

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STOP Solution d'Arrêt Stopreagenz Solucão de paragem Tampone campione Tampon Echantillons Probenpuffer Solución de parada Aντιδραστήριο διακοπής αντίδρασης Sample buffer Tampón Muestras Probenpuffer " Ταμθοι τικό διάλυμα δειγμάτων	CON+ CON- CAL RC CONJ MP WASHB 50x	Controllo positivo Contrôle Positif Positiv Kontrolle Controlo positivo Controllo negativo Controllo negativo Controllo negativo Controlo negativo Calibratore Etalon Kalibrator Calibrador Recupero Corrélation Wiederfindung Recuperacão Conjugát Conjugát Conjugát Micropiastra rivestita Microplacya esensibilisée Beschichtete Mikrotiterplatte Microplaca revestida Tampone di lavaggio Tampon de Lavage Waschpuffer Solucão de lavagem Tampone substrato Substrat Substrat	"Control Positivo "Θετικός ορός ελέγχου "Negative Control "Control Negativo "Αρνητικός ορός ελέγχου "Calibrator "Calibrator "Aντιδραστήριο βαθμονόμησης "Recovery "Recuperado "Ανάκτηση "Conjugate "Conjugate "Conjugato "Σύξευγμα "Coated microtiter plate "Microplaca sensibilizada "Επικαλυμμένη μικροπλάκα "Wash buffer "Solución de lavado "Pυθμιστικό διάλυμα πλύσης "Substrate buffer "Tampón sustrato
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SB 5x	CON+ CON- CAL 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 "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 Calibrator Aντιδραστήριο βαθμονόμησης * Recovery Recuperado Ανάκτηση * Conjugate Conjugate Conjugato Σύζευγμα * Coated microtiter plate Microplaca sensibilizada "Επικαλυμμένη μικροπλάκα * Wash buffer Solución de lavado "Ρυθμιστικό διάλυμα πλύσης * Substrate buffer "Ταπρόn sustrato "Ρυθμιστικό διάλυμα υποστρώματος "Stop solution "Solución de parada
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Diluente de amostra	CON+ CON- CAL RC CONJ MP WASHB 50x SUB STOP	"Controllo positivo "Contrôle Positif "Positiv Kontrolle "Controlo positivo "Controlo negativo "Controlo negativo "Controlo negativo "Controlo negativo "Calibratore "Etalon "Kalibrator "Calibrador "Recupero "Corrélation "Wiederfindung "Recuperacão "Conjugé "Konjugat "Conjugé "Konjugat "Conjugado "Micropiastra rivestita "Microplaque sensibilisée "Beschichtete Mikrotiterplatte "Microplaque sevestida "Tampone di lavaggio "Tampon de Lavage "Waschpuffer "Solucão de lavagem "Tampone substrato "Reagente bloccante "Solution d'Arrêt "Stopreagenz "Solucão de paragem "Tampone campione	Control Positivo Θετικός ορός ελέγχου * Negative Control Control Negativo Aρνητικός ορός ελέγχου * Calibrator Calibrator Aντιδραστήριο βαθμονόμησης * Recovery Recuperado Aνάκτηση * Conjugate Conjugate Conjugate Microplaca sensibilizada Επικαλυμμένη μικροπλάκα * Wash buffer Solución de lavado Pυθμιστικό διάλυμα πλύσης * Substrate buffer Tampón sustrato Pυθμιστικό διάλυμα υποστρώματος Stop solution Solución de parada Aντιδραστήριο διακοπής αντίδρασης Sample buffer Tampón Muestras
	CON+ CON- CAL 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 Conjugát Conjugát Konjugat Conjugát Micropiastra rivestita Microplacu e sensibilisée Beschichtete Mikrotiterplatte Microplaca revestida Tampon de Lavage Waschpuffer Solucão de lavagem Tampone substrato Substrat Substrat Reagente bloccante Solution d'Arrêt Stopreagenz Solucão de paragem Tampone campione	Control Positivo Θετικός ορός ελέγχου * Negative Control * Control Negativo Aρνητικός ορός ελέγχου * Calibrator Calibrator Aντιδραστήριο βαθμονόμησης * Recovery Recuperado Aνάκτηση * Conjugate Conjugate Conjugate Microplaca sensibilizada Επικαλυμμένη μικροπλάκα * Wash buffer Solución de lavado Pυθμιστικό διάλυμα πλύσης * Substrate buffer Tampón sustrato Pυθμιστικό διάλυμα υποστρώματος Stop solution Solución de parada Aντιδραστήριο διακοπής αντίδρασης Sample buffer Tampón Muestras