Version 13-08/24

User's Manual

ASCA IgA/IgG ELISA Kit

REF **DEIA-JY2419**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

ASCA IgG/IgA ELISA is a test system for the quantitative measurement of IgG and IgA class antiSaccharomyces cerevisiae antibodies (ASCA) in human serum or plasma. Accurate diagnosis of inflammatory bowel disease (IBD), in particular the differentiation between the ulcerative colitis and Crohn's disease, is important for treatment and prognosis. ASCA are strongly associated to Crohn's disease. IgG as well as IgA ASCA show a high specificity for Crohn's disease, and support the differential dignosis of IBD, when combined with other serological markers and clinical tests

Principles of Testing

Highly purified mannan from Saccharomyces cerevisiae is bound to microwells.

The determination is based on an indirect enzyme linked immune reaction with the following steps: Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigencomplexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

Reagents And Materials Provided

1. **SORB MT** 1x divisible microplate consisting of 12 modules of 8 wells each. Ready to use.
2. **CAL A-F** 6x 1.5 ml Calibrator A-F (0, 6.3, 12.5, 25, 50, 100 U/ml), containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
3. **CONTROL 1 & 2** 2x 1.5 ml Control positive (1) and negative (2), containing ASCA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
4. **SAM DIL** 5x 20 ml Sample Buffer, containing PBS, BSA, detergent, preservative NaN3 0.09%, yellow, 5x conc.
5. **ENZ CONJ** IgG 15 ml Enzyme Conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.
6. **ENZ CONJ** IgA 15 ml Enzyme Conjugate; containing anti-human IgA antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.
7. **SUB TMB** 15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
8. **STOP SOLN** 15 ml Stop solution; contains acid. Ready to use.
9. **WASH SOLN** 50x 20 ml Wash Buffer, containing Tris, detergent, preservative NaN3 0.09%; 50x conc.
10. 1 Instruction for Use
11. 1 Certificate of Analysis

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
2. Data reduction software
3. Multi-channel dispenser or repeatable pipette for 100 µl
4. Vortex mixer
5. Pipettes for 10 µl, 100 µl and 1000 µl
6. Laboratory timing device
7. Distilled or deionised water
8. Measuring cylinder for 1000 ml and 100 ml
9. Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system.

Storage

1. Store test kit at 2-8°C in the dark.
2. Do not expose reagents to heat, sun, or strong light during storage and usage.
3. Store microplate sealed and desiccated in the clip bag provided.
4. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
5. Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Specimen Collection And Preparation

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.

Reagent Preparation

Wash Buffer

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer

Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well.

Note: Calibrators / Controls are ready to use and need not be diluted.

Assay Procedure

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots and products.
3. All materials must be at room temperature (20-28°C) prior to use.
4. Prepare all reagents and samples. Once started, perform the test without interruption.
5. Double determinations may be done. By this means pipetting errors may become obvious.
6. Perform the assay steps only in the order indicated.
7. Always use fresh sample dilutions.
8. Pipette all reagents and samples into the bottom of the wells.
9. To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
10. Wash microwells thoroughly and remove the last droplets of Wash Solution.
11. All incubation steps must be accurately timed.
12. Do not re-use microplate wells.

TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature
9. Add 100 µl of stop solution to each well of the modules
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690nm) and calculate the results.

The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								
	IgG	IgG	IgA	IgA								

P1, ... patient sample A-F calibrators C+, C- controls

Quality Control

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test

kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

Calculation

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.

Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Performance Characteristics

Calibration

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is

IgG: 0 - 100 U/ml IgA: 0 - 100 U/ml

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay:

Cut-off: IgG-IgA, 10 U/ml-10 U/ml

Interpretation of results - IgG/IgA

Negative: < 10 U/ml

Positive: ≥ 10 U/ml

IgG

Sensitivity: 75.0 %

Specificity: 96.9 %

Overall agreement: 89.1 %

IgA

Sensitivity: 62.5 %

Specificity: 97.7 %

Overall agreement: 85.1 %

Detection Range

6.3 - 100 U/ml

Sensitivity

Functional sensitivity was determined to be:

IgG: 0.5 U/ml

IgA: 0.5 U/ml

Specificity

NA

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E %
IgG 1	1:100	64.2	64.2	100
	1:200	34.1	32.1	106
	1:400	16.9	16.1	105
	1:800	7.6	8.0	95
IgG 2	1:100	53.9	53.9	100
	1:200	26.4	27.0	98
	1:400	12.9	13.5	96
	1:800	7.1	6.7	106
IgG 3	1:100	48.3	48.3	100
	1:200	25.9	24.2	107
	1:400	13.3	12.1	110
	1:800	6.8	6.0	113
IgA 1	1:100	64.2	64.2	100
	1:200	34.1	32.1	106
	1:400	16.9	16.1	105
	1:800	7.6	8.0	95
IgA 2	1:100	53.9	53.9	100
	1:200	26.4	27.0	98
	1:400	12.9	13.5	96
	1:800	7.1	6.7	106
IgA 3	1:100	48.3	48.3	100
	1:200	25.9	24.2	107
	1:400	13.3	12.1	110
	1:800	6.8	6.0	113

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay IgG		
Sample	Mean U/ml	CV %
1	9.6	4.3
2	19.3	6.6
3	76.5	8.8

Inter-Assay IgG		
Sample	Mean U/ml	CV %
1	10.5	7.1
2	31.2	3.8
3	67.3	7.5

Intra-Assay IgA		
Sample	Mean U/ml	CV %
1	5.1	5.2
2	26.8	6.1
3	66.4	6.1

Inter-Assay IgA		
Sample	Mean U/ml	CV %
1	5.9	6.6
2	29.1	6.0
3	81.2	6.4

Interferences

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides)

sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Precautions

1. All reagents of this kit are intended for professional use only.
2. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
4. Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
5. Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
6. Calibrators, Controls, sample buffer and Wash buffer contain sodium azide (NaN3) 0.09% as preservative. This concentration is classified as non-hazardous.
7. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

8. First aid measures: In case of skin contact, immediately wash thoroughly with water and soap.

   Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
9. Personal precautions, protective equipment and emergency procedures:

   Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
10. Exposure controls / personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
11. Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
12. For disposal of laboratory waste the national or regional legislation has to be observed.

    Observe the guidelines for performing quality control in medical laboratories by assaying control sera.