ASCA IgA ELISA Kit

Catalog Number KA1076

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The ASCA IgA ELISA Kit is used for the quantitative and semi-quantitative determination of IgA antibodies to *Saccharomyces cerevisiae* in human serum.

Background

Non-specific inflammatory bowel diseases including Crohn's disease (Enteritis regionalis) and ulcerative colitis (UC) are characterized by unknown etiology as well as chronic-remitting inflammatory processes of the intestine. Whereas the inflammation of ulcerative colitis is restricted to the mucosa and submucosa of colon and rectum, Crohn's disease (CD) shows a wide spread inflammation of the gastro-intestinal tract with granuloma formation.
The risk developing one of these diseases is strongly influenced by immunologic, genetic, infectious and environmental factors.
The differential diagnosis of inflammatory bowel diseases to chronic diarrhea, recurrent abdominal dolor, infectious colitis, anorexia as well as the differentiation of CD to ulcerative colitis is still a high challenge.
The determination of IgA and IgG antibodies to *Saccharomyces cerevisiae* (baker's yeast) has been described as one important serological marker for the differential diagnosis of Crohn's disease recently. Up to 70 % of patients with CD show antibody levels to *Saccharomyces cerevisiae*. Although the cause for their occurrence has been unclear, antibodies to *Saccharomyces cerevisiae* (ASCA) are strongly associated with inflammatory processes of the intestine. In combination with the detection of autoantibodies to atypical anti-neutrophil cytoplasmic antigens (aANCA) which are mainly found in patients with ulcerative colitis, ASCA are a valid parameter for the differentiation of Crohn's disease and ulcerative colitis.

Principle of the Assay

ASCA IgA ELISA Kit is an enzyme immunoassay for the quantitative determination of IgA antibodies to *Saccharomyces cerevisiae* in human serum.

Autoantibodies of the diluted samples, the control, and calibrators react with mannan (cell surface component of baker's yeast) immobilized on the solid phase of a microtiter plate. ASCA IgA guarantees the specific binding of anti-Saccharomyces cerevisiae IgA antibodies of the specimen under investigation by employing purified mannan of *Saccharomyces cerevisiae* for coating. Following an incubation period of 60 min at room temperature, unbound sample components are removed by a wash step.

The bound IgA antibodies react specifically with anti-human-IgA conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method is also possible.

General Information

Materials Supplied

List of component

Component	Amount
Microtiter plate:12 breakable strips per 8 wells coated with mannan (*Saccharomyces cerevisiae*), vacuum sealed with desiccant.	96 (8x12) wells
Concentrated wash buffer: Sufficient for 1000 mL solution each, capped white.	100 mL
Sample diluents: Ready for use, capped black.	100 mL
Conjugate: Containing anti-human-IgA-(sheep) coupled with HRP, ready for use, capped purple.	15 mL
Substrate: 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide, ready for use, capped blue.	15 mL
Stop solution: 0.5 N acidic solution, ready for use, capped yellow.	15 mL
Calibrators (0-4): Diluted serum, ready for use, capped white (conc.: 1, 10, 30, 100, 300 U/mL).	1 mL x 5
Positive control: Diluted serum, ready for use, capped red.	1 mL
Negative control: Diluted serum, ready for use, capped green.	1 mL

Storage Instruction

- ✓ The expiry date of each component is reported on its respective label that of the complete kit on the box labels.
- ✓ Upon receipt, all components of the ASCA IgA have to be kept at 2-8°C, preferably in the original kit box.
- ✓ After opening all kit components are stable for at least 2 months, provided proper storage.

Materials Required but Not Supplied

- ✓ Micropipette 100-1000 µL
- ✓ Micropipette 10-100 µL
- ✓ Multi-channel pipette 50-200 µL
- ✓ Trough for multi-channel pipette
- ✓ 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- ✓ Microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- ✓ Graduated cylinders
- ✓ Distilled or de-ionized water

Precautions for Use

- ✓ This kit is for in vitro use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only.
- ✓ The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- ✓ Do not use or mix reagents from different lots.
- ✓ Do not use reagents from other manufacturers.
- ✓ Avoid time shift during pipetting of reagents.
- ✓ All reagents should be kept at 2-8°C before use in the original shipping container.
- ✓ Some of the reagents contain small amounts of Neolone (1.0% v/v) as preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.
- ✓ Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- ✓ Since the kit contains potentially hazardous materials, the following precautions should be observed:
- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

Assay Protocol

Reagent Preparation

- ✓ Allow all components to reach room temperature prior to use in the assay.
- ✓ The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells.
- ✓ Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
- ✓ Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1+9) with deionized or distilled water. For example, dilute 8 mL of the concentrate with 72 mL of distilled water. The wash solution prepared is stable up to 30 days at 2-8°C.
- ✓ Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.
- ✓ Avoid exposure of the TMB substrate solution to light.

Sample Preparation

- ✓ Specimen collection and storage

- Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic and contaminated samples should not be used.
- The samples may be kept at 2-8°C for up to three days. Long-term storage requires -20°C.
- Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20°C.

- ✓ Preparation before use

  Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Samples have to be diluted 1 + 100 (v/v), e.g. 10 µL sample + 1.0 mL sample diluent, prior to assay.

Assay Procedure

- ✓ Dilute patient sera with sample diluents 1 + 100 (v/v), e.g. 10 µL serum + 1.0 mL sample diluent.
- ✓ Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µL calibrators 1-4 (calibrator 0 optionally, quantitative) or
   100 µL calibrator 1 (semi-quantitative)
   100 µL positive control
   100 µL diluted samples into the respective wells.
3. Cover plate, incubate 60 min at room temperature (18°C-25°C).
4. Decant, then wash each well three times using 300 µL wash solution.
5. Add 100 µL of conjugate solution to each well.
6. Cover plate, incubate 30 min at room temperature (18°C-25°C).
7. Decant, then wash each well three times using 300 µL wash solution.
8. Add 100 µL of substrate to each well.
9. Cover plate, incubate 15 min protected from light at room temperature (18°C-25°C).
10. Add 100 µL of stop solution to each well and mix gently.
11. Read the OD at 450 nm versus 620 or 690 nm within 30 min after adding the stop solution.

Data Analysis

Calculation of Results

ASCA IgA ELISA Kit allows both the quantitative and semi-quantitative evaluation of the results.

✓ Qualitative evaluation

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the calibrators 1-4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective ASCA IgG-concentrations on the abscissa, x-axis, (log. scale). Anti-Saccharomyces cerevisiae concentrations of the unknown samples are directly read off in U/mL against the respective OD values. Using the recommended dilution of 1 + 100 (v/v) for sample sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

✓ Semi-quantitative evaluation

Results can be calculated semi-quantitatively calculating the binding index BI (ratio) between the optical density of an unknown sample and the optical density of calibrator 1 (10 U/mL) multiplied by a factor 2.

$$BI = OD_{sample} / (OD_{calibrator\ 1\ (10\ U/mL)} \times 2)$$

Both evaluation variants of ASCA IgA ELISA Kit may be achieved also with computer assisted analysis software intergrated in the photometers.

✓ Example of typical assay results (quantitative)

wells	OD (a)	OD (b)	OD (mean)	U/mL
Calibrator 0	0.107	0.088	0.098	1
Calibrator 1	0.238	0.306	0.272	10
Calibrator 2	0.604	0.618	0.611	30
Calibrator 3	1.480	1.515	1.498	100
Calibrator 4	2.620	2.595	2.608	300
Sample 1	1.192	1.204	1.198	73

Note: The above mentioned calibrator concentrations are only an example for a typical standard curve. They can change from lot to lot.

✓ Test validity

The test run is valid if:

The mean OD of the calibrator 1 is ≤ 0.5

The mean OD of the calibrator 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps

etc.). In case of repeated failure of the quality criteria contact the supplier.

✓ Reference Values

ASCA IgA ELISA Kit	U/mL	BI
positive	≥ 20	≥ 1.0
negative	< 20	< 1.0

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum ASCA IgA antibody levels as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

✓ Limitations of Method

Healthy individuals should be tested negative by the ASCA IgA ELISA Kit. However, ASCA IgA antibody positive apparently healthy persons do occur.

Performance Characteristics

✓ Calibration

Due to the lack of an international reference material the ASCA IgA ELISA Kit is calibrated in arbitrary units (U/mL).

✓ Detection sensitivity and specificity

The detection sensitivity and specificity of ASCA IgA, were determined by testing 116 individuals with Chrohn's disease and 75 individuals with ulcerative colitis.

Sensitivity: 42%

Specificity: 92%

✓ Frequency distribution

50 normal sera (without clinical symptoms) were tested ASCA IgA. All serum was found with concentration above the cut-off (20 U/mL). This corresponds to a specificity of 100 %.

✓ Precision

Intra-assay Variance (n=20)		Inter-assay Variance (n=5 x10)	
U/mL	CV (%)	U/mL	CV (%)
199	6.6	215	8.2
65	4.4	65	6.8
18	6.7	19	8.6

Resources

References

1. Conrad K, Schmechta H, Klafki A, Lobeck G, Uhlig HH, Gerdi S, Henker J: Serological differentiation of inflammatory bowel diseases. Eur J Gastrol & Hepatol. 2002 14:129-135.
2. Vermeire S: Serological Diagnosis in IBD. IBDM 2002 3:82-89.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Calibrator A	Sample 2										
B	Calibrator B	Sample 3										
C	Calibrator C											
D	Calibrator D											
E	Calibrator E											
F	Control +											
G	Control -											
H	Sample 1											