Human ASCA IgG / IgA ELISA Kit

Enzyme Immunoassay for the quantification of IgG and IgA anti-Saccharomyces cerevisiae antibodies (ASCA) in human serum or plasma.

Catalog number: ARG80370

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INTRODUCTION

Accurate diagnosis of inflammatory bowel disease (IBD), in particular the differentiation between the two major IBDs ulcerative colitis and Crohn's disease, is important for treatment and prognosis. Ulcerative colitis is characterized by an inflammation and ulcers in the top layers of the lining of the colon and rectum. Crohn's disease shows a wide spread inflammation of the gastro-intestinal tract with granuloma formation extending deep into the affected tissue. Inflammation in Crohn's disease is asymmetrical and segmental, with areas of both healthy and diseased tissue, in contrast to ulcerative colitis where inflammation is symmetrical and uninterrupted from the rectum proximally [1].

To differentiate between Crohn's disease and ulcerative colitis the detection of ANCA (Anti-Neutrophil Cytoplasmic Antibody) and ASCA (Anti-Saccharomyces Cerevisiae Antibody) can be used. ASCA are directed against oligomannosidic epitopes on the cell wall mannan (phosphor-peptidomannan) of the yeast Saccharomyces cerevisiae [2]. IgG as well as IgA ASCA show a specificity of 95-100% for Crohn's disease. ASCA are strongly associated to Crohn's disease. Stud-ies showed 5% positive IgG and 7% IgA class ASCA in ulcerative colitis whereas in Crohn's disease a sensitivity of 75% for IgG and 60% for IgA class ASCA could be observed [3, 4]. The occurrence of atypical ANCA (aANCA) in Crohn's disease is more infrequent than in ulcerative colitis. The prevalence of ANCA varies from 50% to 90% in ulcerative colitis and 10% to 20% in Crohn's disease [5]. The combination of both serological tests makes possible a rapid and non-invasive differential diagnosis between Crohn's disease and ulcerative colitis.

1. Merck Manual of Diagnosis and Therapy. 1999. Whitehouse Station, N. J. (www.merck.com/pubs/mmanual).

2. Sendid, B., J. F. Colombel, P. M. Jacquinot, C. Faille, J. Fruit, A. Cortot, D. Lucidarme, D. Camus, and D. Poulain. Specific antibody response to oligomannosidic epitopes in Crohn's Disease. Clin. Diag. Lab. Immunol., 1996, 3(2): 219-226.

3. Main, J., H. McKenzie, G. R. Yeaman, M. A. Kjerr, D. Robson, and C. R. Pennington. Antibody to Saccharomyces cerevisiae (baker´s yeast) in Crohn's disease. Brit. J. Med., 1988, 297: 1105- 1106.

4. McKenzie, H., J. Main, C. R. Pennington, and D. Parrat. Antibody to selected strains of Saccharomyces cerevisiae (baker´s and brewer´s yeast) and Candida albicans in Crohn's disease. Gut, 1990, 31:536-538.

5. Quinton, J.-F., B. Sendid, D. Reumaux, P. Duthilleul, A. Cortot, B. Grandbastien, G. Charrier, S. R. Targan, J.-F. Colombel, and D. Poulain. Anti-Saccharomyces cerevisiae mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut, 1998, 42: 788-791.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A highly purified mannan from Saccharomyces cerevisiae has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated human IgA or IgG antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion

to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard 0-5 (0, 6.3, 12.5, 25, 50, 100 U/ml)	6 X 1.5 ml (Ready-to-use)	4°C.
Control 1 (Positive control) (40 U/ml; Acc. Range 30-50 U/ml)	1 X 1.5 ml (Ready-to-use)	4°C
Control 2 (Negative control) (2 U/ml; Acc. Range <5 U/ml)	1 X 1.5 ml (Ready-to-use)	4°C
5X Sample buffer	20 ml	4°C
HRP-conjugated Human IgA Antibody	15 ml (Ready-to-use)	4°C
HRP-conjugated Human IgG Antibody	15 ml (Ready-to-use)	4°C
50X Wash buffer	20 ml	4°C
TMB substrate	15 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	15 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 620 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C in dark at all times.
- Diluted Wash buffer and sample buffer could be stored at 4 °C at least one month. We recommend consumption on the same day.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Prepare all reagents and samples before usage. Once started, perform the test without interruption.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

- Avoid using hemolysis or lipemia samples, although it might not interfere with this assay.
- Heat-inactivated sera is not recommended for this assay.

REAGENT PREPARATION

- 1X Wash buffer: Prior to use, dilute 50X Wash buffer (50 ml) into distilled water (950 ml) to yield 1X Wash buffer. The diluted washing solution is stable for 4 weeks at 4°C. We recommended preparing freshly at the same day.
- 1X Sample buffer: Prior to use, dilute 5X Sample buffer (20 ml) with distilled water (80 ml) to yield 1X Sample buffer. The diluted sample solution is stable for 4 weeks at 4°C. We recommended preparing freshly at the same day.
- Patient sample: Dilute patient sample 1:100 before assay by adding 10 µl of sample into 990 µl 1X sample buffer, mix well.

  (Note: Standards and Controls are ready to use and need not be diluted.)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-28 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of standards, controls and diluted samples into wells.
3. Incubate for **30 minutes at RT**.
4. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes**. Wash by filling each well with **1× Wash Buffer (300µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **100 µl** of **HRP-conjugated Human IgA antibody (or HRP-conjugated Human IgG antibody)** into each well. Incubate for **15 minutes at RT**.
6. **Wash** as according to **step 4.**
7. Add **100 µl** of **TMB Substrate Reagent** to each well. Incubate for **15 minutes at RT**.
8. Add **100 µl** of **Stop Solution** to each well. Incubate for **5 minutes at RT**. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at **450nm immediately**. (*Optional: read at 620nm or 600-690 nm as reference wavelength*). The developed color is stable for at least 30 minutes. Read the OD within 30 minutes after adding stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

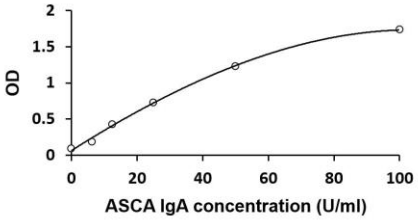
2. Using lin-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

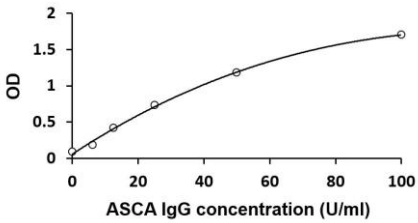
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.





INTERPRETATION OF RESULTS

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 for IgG/IgA: 10 U/ml

IgG: Negative: <10 U/ml, Positive: ≥ 10 U/ml

IgA: Negative: <10 U/ml, Positive: ≥ 10 U/ml

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of ASCA IgG/IgA ranged from 6.3-100 U/ml. The mean MDD was 0.5 U/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.5% and inter-assay precision was 6.1%. [IgG]

The CV value of intra-assay precision was 5.8% and inter-assay precision was 6.3%. [IgA]

Linearity

IgG: 95-113%

IgA: 95-113%