11. Assay Procedure Continued…

9. Add 100µl of Stop Solution **(Reagent 5)** to each well. To allow equal reaction times, the Stop Solution should be added to the wells in the same order as the TMB Substrate.

10. Read the optical density (OD) of each well at 450nm in a microplate reader within 10 minutes. A 620nm filter may be used as a reference wavelength.

12. Quality Control

Quality control data is supplied on the lot-specific QC certificate included in the kit.

Controls are intended to monitor for substantial reagent failure.

Any well positive by spectrophotometer but without visible colour should be cleaned on the underside and re-read. If OD values below zero are observed, the wavelengths used should be verified, the reader re-blanked to air and the measurements repeated.

13. Interpretation of Results

Semi-Quantitative Results

Plot the ODs of the 0 U/ml and 10 U/ml standard against concentration and join the points. Alternatively, a point-to-point curve-fitting algorithm can be applied using appropriate computer software. The concentrations of unknowns can then be determined from this graph.

Qualitative Results

Samples with OD < 10 U/ml Standard have normal ASCA levels.
Samples with OD > 10 U/ml Standard have elevated ASCA levels.

14. Limitations of the Procedure

1. A normal ASCA result does not rule out the presence of Crohn's disease.
2. An elevated ASCA level does not necessarily indicate the presence of Crohn's disease.
3. The assay performance has not been established for paediatric Crohn's disease or ulcerative colitis patients.

15. Performance Characteristics

Clinically defined specimens from patients with Crohn's disease, ulcerative colitis, hepatitis C, autoimmune hepatitis, celiac disease and from healthy control subjects were tested with the Genesis ASCA IgG & IgA ELISA kits. The results are given in the table below.

Condition	n	IgA Neg	IgA Pos	IgG Neg	IgG Pos	IgA & IgG Pos
Crohn's	92	3	89	10	82	92
Ulcerative colitis	88	75	13	71	17	22
Healthy Controls	93	86	7	84	9	0
Hep C +ve	10	10	0	10	0	0
Autoimmune hepatitus	9	9	0	9	0	0
Coeliac disease	9	5	4	6	3	4

16. Reproducibility

Within Assay Precision

Three specimens were tested a total of sixteen times each within a single assay.

Mean OD	n	SD	CV%
0.44	3	0.01	2.27
0.68	3	0.02	2.94
1.38	3	0.03	2.17

Between Assay Precision

Three specimens were tested in duplicate on six independent occasions.

Mean OD	n	SD	CV%
0.62	3	0.01	4.83
1.06	3	0.07	6.60
1.67	3	0.09	5.39

17. Method Summary

- Dilute sera 1:50 with Sample Diluent **(Reagent 1)**
- Dispense 100µl of Sample Diluent (0 U/ml), the 10 U/ml Standard, the Positive and Negative Controls and the diluted sample into the microplate wells
- Incubate for **30 minutes** at room temperature.
- *Wash the wells three times*
- Dispense 100µl of Conjugate **(Reagent 3)** into each well
- Incubate at room temperature for **30 minutes**
- *Wash the wells four times*
- Add 100µl of TMB Substrate **(Reagent 4)** to each well
- Incubate at room temperature for **10 minutes**
- Add 100µl Stop Solution **(Reagent 5)** to each well
- Read the optical density at 450nm (single wavelength) or
- 450/620nm (dual wavelength).

18. Further Reading

Hoffenberg EJ *et al*. Serologic testing for inflammatory bowel disease. J. Pediatr. 1999; 134: 447-52.

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Sendid B *et al*. Anti-*Saccharomyces cerevisiae* mannan antibodies in familial Crohn's disease. Am. J. Gastroenterol. 1998; 93: 1306-10.

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G·E·N·E·S·I·S Diagnostics

ASCA IgG ELISA Kit

Qualitative/semi-quantitative assays for the detection of *Saccharomyces cerevisiae* IgG antibodies

Product Code: GD078

For *in vitro* Diagnostic Use

CE

1. Materials Included in the Kit

- **Microplate:** 96 wells in 12 X 8 break-apart strips, pre-coated with purified mannan, with holder in a foil bag with desiccant
- **Reagent 1**: **Sample Diluent** 10mM Tris-buffered saline, pH 7.2 with antimicrobial agent 50ml, (blue), ready to use
- **Reagent 2: Wash Buffer** 100mM Tris-buffered saline with detergent, pH 7.2, 100 ml, **concentrate** (x10)
- **Reagent 3: Conjugate**: rabbit anti-human IgG (red) conjugated to horseradish peroxidase in protein stabilising solution and antimicrobial agent, 12 ml, ready to use
- **Reagent 4: TMB Substrate** aqueous solution of TMB and hydrogen peroxide, 12 ml, ready to use
- **Reagent 5: Stop Solution** 0.25M sulphuric acid, 12 ml, ready to use
- **Standard:** 10 U/ml**,** 1ml of 10mM Tris-buffered saline containing human serum antibodies to *S.cerevisiae*, ready to use
- **Positive Control:** 1ml of 10mM Tris-buffered saline containing human serum antibodies to *S.cerevisiae*, ready to use
- **Negative Control:** 1ml of 10mM Tris-buffered saline containing normal human serum, ready to use.
- **Instructions for use**

263-078-04

2. Other Equipment Required

Test tubes for dilution • graduated cylinder for preparing wash buffer • precision pipettes and disposable tips to deliver 10µl, 100µl, 1ml • EIA microplate washer or multi-channel pipette or wash bottle • distilled or de-ionised water • absorbent paper • EIA microplate reader with 450nm and optional 620nm reference filter. Alternatively, a suitable, self-validated automated system may be used.

Instrumentation, whether manual or automated, should meet the following criteria: pipettes with better than 3% imprecision with no carry over between pipetting steps; microplate washers should remove 99% of fluid; automated machines should minimise time between washing and adding the next reagent.

3. Intended Use

The ASCA IgG kit is a rapid ELISA method for the qualitative/semi-quantitative detection of anti-*Saccharomyces cerevisiae* antibodies (ASCA). It is intended as an aid to the diagnosis of Crohn's disease.

4. Explanation of the Test

Inflammatory bowel diseases (IBD), which include Crohn's disease and ulcerative colitis, occur worldwide and are characterised by unknown aetiology and chronic intestinal inflammation. Diagnosis comprises mainly colonoscopy and X-ray.

Antibodies to oligomannosidic epitopes of the baker's yeast S. cerevisiae have been shown to be strongly associated with inflammatory processes of the intestine. Importantly, IgA and IgG ASCA have been described as important serological markers for the diagnosis of Crohn's disease. Approximately 60% of patients with Crohn's disease have been found to be positive for IgG antibodies to S.cerevisiae, whereas the incidence in patients with ulcerative colitis is no different from that in healthy control subjects.

Perinuclear anti-neutrophil cytoplasmic autoantibodies (pANCA), which occur infrequently in patients with Crohn's disease, are a recognised marker for ulcerative colitis. Thus, serological testing for pANCA in combination with ASCA can assist in the differential diagnosis of IBD and the selection of appropriate therapies.

5. Principle of the Test

Diluted serum samples are incubated with mannan immobilised on microtitre wells. After washing away unbound serum components, rabbit anti-human IgG conjugated to horseradish peroxidase is added to the wells, and this binds to surface-bound antibodies in the second incubation. Unbound conjugate is removed by washing, and a solution containing 3,3',5,5'-tetramethylbenzidine (TMB) and enzyme substrate is added to trace specific antibody binding. Addition of Stop Solution terminates the reaction and provides the appropriate pH for colour development. The optical densities of the 10 U/ml standard, controls and samples are measured using a microplate reader at 450nm. A sample is considered positive if the optical density is greater than that of the 10 U/ml standard.

6. Safety Precautions

1. All reagents in this kit are for *in vitro* diagnostic use only.
2. Only experienced laboratory personnel should use this test. The test protocol must be followed strictly.
3. CAUTION: the device contains material of human and animal origin and should be handled as a potential transmitter of diseases. All human source material used in the preparation of standards and control for this product have been tested and found negative by ELISA for antibodies to HIV, HbsAg and HCV. No test method, however, can offer complete assurance that infectious agents are absent. Therefore, all reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
4. Reagents of this kit contain antimicrobial agents and the Substrate solution contains 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
5. The Stop Solution contains 0.25M sulphuric acid. Avoid contact with skin and eyes. Rinse immediately with plenty of water if contact occurs.
6. Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Dispose of plates and specimens as clinical waste. Any unused reagents should be flushed away with copious amounts of water. Disposal must be performed in accordance with local legislation.

7. Technical Precautions

1. Strips and solutions should not be used if the foil bag is damaged or liquids have leaked.
2. Allow all reagents and the microplate to reach room temperature before use. Ensure that the microplate foil bag containing any unused strips is well sealed and contains the desiccant to avoid moisture. Store at 2 - 8ºC after use.
3. Include the Positive and Negative Control in every test run to monitor for reagent stability and correct assay performance.
4. Strictly observe the indicated incubation times and temperature.
5. When automating, consider excess volumes required for setting up the instrument and dead volume of robot pipette
6. Ensure that no cross-contamination occurs between wells. Keep all pipettes and other equipment used for Conjugate completely separate from the TMB Substrate reagent.
7. When pipetting Conjugate or TMB Substrate, aliquots for the required numbers of wells should be taken to avoid multiple entry of pipette tips into the reagent bottles. Never pour unused reagents back into the original bottles.
8. Do not allow microwells to dry between incubation steps.
9. Strictly follow the described wash procedure. Insufficient washing may cause high background signal.
10. Avoid direct sunlight and exposure to heat sources during all incubation steps.
11. Replace colour-coded caps on their correct vials to avoid cross-contamination
12. It is important to dispense all samples and controls into the wells without delay. Therefore ensure that all samples are ready to dispense.

8. Shelf Life and Storage Conditions

On arrival, store the kit at 2 - 8°C. Once opened the kit is stable for 3 months (or until its expiry date if less than 3 months). Do not use kits beyond their expiry date. Do not freeze any kit component. The diluted Wash Buffer has a shelf life of 3 months if stored in a closed bottle at 2 - 8ºC.

9. Specimen Collection and Storage

Serum and plasma samples may be used and should be stored at -20°C for long-term storage. Frozen samples must be mixed well after thawing and prior to testing. Repeated freezing and thawing can affect results. Addition of preservatives to the serum sample may adversely affect the results. Microbially contaminated, heat-treated or specimens containing particulate matter should not be used. Grossly haemolysed, icteric or lipaemic specimens should be avoided.

10. Preparation of Reagents

Dilute the Wash Buffer **(Reagent 2)** 1:9 in distilled water to make sufficient buffer for the assay run e.g. add 50ml wash buffer concentrate to 450ml water.

11. Assay Procedure

1. Dilute patient samples 1:50 in Sample Diluent **(Reagent 1)** (e.g. 10µl serum plus 0.5 ml diluent).

2. Assemble the number of strips required for the assay.

3. For qualitative assays, dispense the 10 U/ml Standard, the Negative and Positive Controls and diluted patients samples. For semi-quantitative assays, dispense 100 µl of Sample Diluent as the 0 U/ml standard followed by the 10 U/ml Standard, the Positive and Negative Control and the diluted patient sample into appropriate wells.

4. Incubate for **30 minutes** at room temperature.

5. After 30 minutes, decant or aspirate the well contents and wash the wells 3 times using automated washing or the manual wash procedure (see below). Careful washing is the key to good results. **Do not allow the wells to dry out.**

   Manual Wash Procedure
   Empty the wells by inversion. Using a multi-channel pipette or wash bottle, fill the wells with Wash Buffer. Empty by inversion and blot the wells on absorbent paper. Repeat this wash process 2 more times.

6. Dispense 100µl of Conjugate **(Reagent 3)** into each well. Incubate the wells for **30 minutes** at room temperature.

7. After 30 minutes, discard the well contents and carefully wash the wells 4 times with Wash Buffer. Ensure that the wells are empty but do not allow to dry out.

8. Using a repeating dispenser, rapidly dispense 100µl of TMB Substrate **(Reagent 4)** into each well. Incubate the plate for **10 minutes**.