DRG® ASCA combi ELISA (EIA-4278)

REVISED 16 JUNE 2005 RM (VERS. 1.1) **RUO IN THE USA**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

ASCA combi is used for measurement of both IgG and IgA antibodies to Saccharomyces cerevisiae in human serum.

DRG offers innovative assays for measurement of inflammatory bowl diseases: ASCA IgA, ASCA IgG and ASCA combi. All assays employ the same assay scheme and predilution maximizing laboratory efficiency

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

PRINCIPLE OF THE TEST

ASCA combi is an enzyme immunoassay for measurement of both IgG and IgA antibodies to Saccharomyces cerevisiae in human serum.
Autoantibodies of the diluted specimen samples and controls react with mannan (cell surface component of baker's yeast) immobilized on the solid phase of a microtiter plate. ASCA combi guarantees the specific binding of anti-Saccharomyces cerevisiae IgG as well as IgA antibodies of the specimen under investigation by employing purified mannan of Saccharomyces cerevisiae for coating. Following an incubation period of 60 min at 37°C, unbound serum components are removed by a washing step.
The bound antibodies react specifically with anti-human-IgG and IgA antibodies conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at 37°C. Excessive conjugate is separated from the solid-phase immune complexes by the following washing step.
HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. This enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 10 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.

SPECIMEN SAMPLES

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.
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: Specimen samples have to be diluted **1 + 50** (v/v),

e.g. 10 µl sample + 0.5 ml sample diluent (C), **prior to assay**.

The samples may be kept at 2-8°C for up to two days. Long-term storage requires -20°C.

TEST COMPONENTS
For 96 determinations

A **Ag 96**	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with mannan (Saccharomyces cerevisiae)	1 vacuum sealed with desiccant
B **BUF** **WASH**	**Concentrated wash buffer** sufficient for 1000 ml solution **10x**	100 ml concentrate capped white
C **DIL**	**Sample diluent**	50 ml ready for use capped black
D **CONJ**	**Conjugate** containing anti-human-IgG and IgA (sheep) coupled with HRP	15 ml ready for use capped red
E **SOLN** **TMB**	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F **H2SO4**	**Stop solution** 0.25 M sulfuric acid **0.25M**	15 ml ready for use capped yellow
P **CONTROL**	**Positive Control** (diluted serum), +	1 ml ready for use
CO **CONTROL**	**Cut-off** (diluted serum), **C**	1 ml each ready for use
N **CONTROL**	**Negative Control** (diluted serum), –	1 ml ready for use

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Materials required
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
incubator (37°C)
microplate reader with optical filters for 450 nm and 620 nm or 690 nm
distilled or de-ionized water

Size and storage
ASCA combi has been designed for 96 determinations.
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 combi 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.

Preparation before use
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 per strip.
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!

ASSAY PROCEDURE
Dilute specimen sera with sample diluent (C) 1 + 50 (v/v),
e.g. 10 µl serum + 0.5 ml sample diluent (C).
Avoid any time shift during pipetting of reagents and samples.

Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
Dispense
100 µl Cut-off Control (CO)
100 µl Controls (P, N))
100 µl diluted specimen samples into the respective wells.
Seal plate, incubate **60 min** at 37°C.
Decant, then wash each well **five times** using **300 µl** wash buffer (made of B).

Add **100 µl** of conjugate (D) solution to each well.
Seal plate, incubate **30 min** at 37°C.
Decant, then wash each well **five times** using **300 µl** wash buffer (B).
Add **100 µl** of substrate (E) to each well.
Incubate **10 min** protected from light at room temperature (18-25°C).
Add **100 µl** of stop solution (F) to each well and mix gently.
Read the optical density at **450 nm** versus 620 or 690 nm **within 30 min** after adding the stop solution.

DATA PROCESSING
Qualitative evaluation
Results are interpreted by calculating the binding index (BI)
BI = $OD_{sample}/OD_{cut\text{-}off\ control}$

This calculation can be done by the integrated evaluation software of the microplate reader used, too.

Example of typical assay results

wells	**OD (a)**	**OD (b)**	**OD** (mean)	**BI**
Positive control	1.965	1.977	1.971	
Cut-off control	0.353	0.371	0.362	
Negative control	0.047	0.048	0.048	
Specimen 1	0.941	0.932	0.937	2.6 - positive
Specimen 2	0.264	0.260	0.262	0.7 - negative
Specimen 3	0.369	0.377	0.373	1.0 - positive

Test validity
The test run is valid if:
the mean OD of the negative control is ≤ 0.150
the mean OD of the positive control is ≥ 1.200
the mean OD of the cut-off control is ≥ 0.200
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 your supplier.

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SAFETY PRECAUTIONS

In the United States, this kit is intended for Research Use Only.

Follow the working instructions carefully. DRG and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. 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 Thimerosal (< 0.1% w/v) and Kathon (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 specimen 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.