DRG® ASCA IgG ELISA (EIA-4280)

CE

RUO in the USA

Revised 29 Dec. 2009 rm (Vers. 4.1)

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

ASCA IgG is for measurement of IgG antibodies to *Saccharomyces cerevisiae* in human serum.

DRG offers two innovative serological markers for inflammatory bowl diseases: **ASCA IgA** and **ASCA IgG**.

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

2 PRINCIPLE OF THE TEST

ASCA IgG is an enzyme immunoassay for measurement of IgG antibodies to *Saccharomyces cerevisiae* in human serum.

Autoantibodies of the diluted specimen 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 IgG guarantees the specific binding of anti-Saccharomyces cerevisiae IgG 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 IgG antibodies react specifically with anti-human-IgG 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.

3 SPECIMEN SAMPLES

3.1 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.

3.2 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 + 100 (v/v),***
e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.

4 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; 2 foils
B **(BUF WASH)** **(10X)**	**Concentrated wash buffer** sufficient for 1000 ml solution each	100 ml concentrate capped white
C **(DIL)**	**Sample diluent**	100 ml ready for use capped black
D **(CONJ)**	**Conjugate** containing anti-human-IgG - (sheep) coupled with horse radish peroxidase	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)** **(0.25M)**	**Stop solution** 0.25 M sulfuric acid	15 ml ready for use capped yellow

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0 – 4	**Calibrators**	1 ml each
(CAL)	(diluted serum)	ready for use
	conc.: 1, 10, 30, 100, 300 U/ml	capped white
P	**Positive control**	1 ml
(CONTROL)	(diluted serum)	ready for use
(+)	conc.: see leaflet enclosed	capped red

4.1 Materials required in addition

- 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

4.2 Size and storage

ASCA IgG 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 IgG 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.

4.3 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 de-ionized 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!

5 ASSAY PROCEDURE

- **Dilute specimen sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C).**
- **Avoid any time shift during pipetting of reagents and samples**

1. Bring all reagents to room temperature (18 °C - 25 °C) before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators 1 – 4 (CAL 0 optionally, quantitative) or
   100 µl calibrator 1 (semi-quantitative)
   100 µl positive control (P)
   100 µl diluted specimen 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 (made of B).
5. Add **100 µl** of conjugate (D) 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 (made of B).
8. Add **100 µl** of substrate (E) 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 (F) 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.

6 DATA PROCESSING

ASCA IgG allows both the quantitative and semi-quantitative evaluation of the results.

6.1 Quantitative 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 specimen's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

6.2 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**.

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

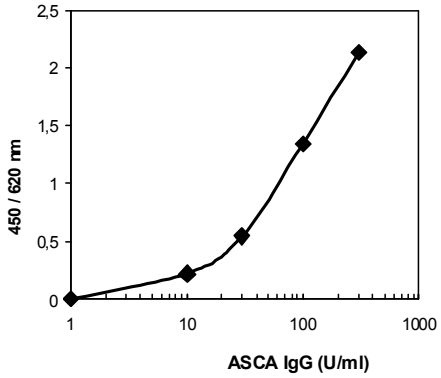
Both evaluation variants of ASCA IgG may be achieved also with computer assisted analysis software intergrated in the photometers.

Example of typical assay results (quantitative)

well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator 0	0.005	0.005	0.005	1
Calibrator 1	0.217	0.223	0.220	10
Calibrator 2	0.537	0.550	0.543	30
Calibrator 3	1.334	1.354	1.344	100
Calibrator 4	2.100	2.162	2.131	300
Sample 1	1.192	1.204	1.198	85

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

TYPICAL STANDARD CURVE (example)



Specimens with an OD > calibrator 4 should be retested in a greater sample dilution. The results have to be multiplied with the chosen dilution factor.

7 INCUBATION SCHEME

Dilute specimens sample★	**10 µl serum + 1.0 ml sample diluent (C)**

★This dilution can also be used in the ASCA IgA (EIA-4279)

			calibrators	control	sera
1	Bring all ready for use reagents to room temperature (18 °C - 25 °C) before use.				
2	Pipette	Calibrators (0 - 4) or Calibrator 1	100 µl		
		Positive Control (P)		100 µl	
		prediluted 1 + 100 specimen sera			100 µl
3	Incubate		60 minutes at room temperature (18 °C - 25 °C)		
4	Wash		Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at room temperature (18 °C - 25 °C)		
7	Wash		Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		15 minutes at room temperature (18 °C - 25 °C)		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

8 SAFETY PRECAUTIONS

- **This kit is 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 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 HIV as well as for 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.