

## Instructions for use

# IBDX® gASCA IgG ELISA Kit

Semi-quantitative ELISA for anti-*Saccharomyces cerevisiae* IgG antibodies in human serum or plasma  
**96 determinations**



Store at 2-8°C  
 Reagents included in the kit are  
 for *in vitro* diagnostic use only







REF	CAT No <b>S701100</b>
IVD	<i>In vitro</i> diagnostic device

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 <b>LOT</b>	Batch number	 <b>REF</b>	Catalogue number
 <b>Manufacturer</b>		 <b>Temperature limitation</b>	
 <b>Expiration date</b>		 <b>Consult operating instructions</b>	

## INTENDED USE

The IBDX gASCA IgG ELISA kit is an indirect solid phase enzyme-linked immunosorbent assay (ELISA) for the detection of circulating IgG class antibodies against *Saccharomyces cerevisiae* antigen in human serum. It is intended as an aid for the diagnosis and management of Crohn's disease (CD). Use of the gASCA IgG kit together with the other IBDX kits is recommended to obtain optimal results.

## INTRODUCTION

Inflammatory bowel disease (IBD) is a general term used to describe diseases that cause inflammation of the intestines. Two prominent classes of IBD are Crohn's disease (CD) and ulcerative colitis (UC), both of which occur worldwide and are characterized by chronic intestinal inflammation of unknown aetiology.

Other gastrointestinal functional disorders (OGD) with symptoms similar to those of IBD are irritable bowel syndrome (IBS) and celiac. IBS is a functional disorder of the colon, a non-inflammatory disease diagnosed only by ruling out all other disease options. Celiac denotes sensitivity to gluten, which is controlled through maintaining a strict diet. Other non-IBD categories are caused by bacterial and viral infections.

Though CD and UC share several symptoms, the course, complications, and management of these diseases considerably differ, in particular regarding their surgical aspects. Consequently, the differential diagnosis of these diseases is pivotal to establish proper treatment.

The major commercially applied serologic markers for IBD are anti-*Saccharomyces cerevisiae* antibodies (ASCA) and atypical peri-nuclear antineutrophil cytoplasmic antibodies (pANCA). ASCA are directed against phosphomannan in the cell walls of the yeast *S. cerevisiae*. Their prevalence amounts to 48%–69% among CD patients and 15% among UC patients (Reumaux, D., et al., 2003; Sendid, B., et al., 1996). pANCA are autoantibodies directed at antigens found mostly in azurophilic granules of neutrophils with a prevalence of up to 85% among UC patients and 5%–28% among CD patients as well as healthy people (Reumaux, D., et al., 2003).

Laminaribioside, Chitobioside and Mannobioside are novel sugar antigens, known as innate immunity triggers. Antibodies recognizing these antigens are novel serologic markers in Crohn's disease, including ASCA-negative CD patients (Dotan, I., 2006). gASCA is an improved ASCA assay based on immobilized purified Mannan polysaccharide. Anti-chitobioside carbohydrate antibodies (ACCA), anti-mannobioside carbohydrate antibodies (AMCA), and anti-laminaribioside carbohydrate antibodies (ALCA) provide high specificity while exploiting a cost effective standard enzyme immunoassay format. Together, ALCA, AMCA and ACCA may identify 33-44% of the ASCA negative Crohn's disease patients (Dotan, I., et al., 2006; Ferrante, M., et al., 2006). A sera positive result with multiple antigens was found to be associated with a more complicated and severe disease course and with a higher risk for abdominal surgery (Ferrante, M., et al., 2006).

Glycominds offers the IBDX panel including gASCA IgG, ALCA IgG, ACCA IgA and AMCA IgG ELISA kits to aid in the diagnosis and management of CD. When used concurrently with pANCA, these kits provide a cost effective, accurate and sensitive tool to aid in the diagnosis of IBD, as well as in the diagnosis and management of Crohn's disease patients.

## PRINCIPLES OF THE PROCEDURE

Diluted serum samples (1:101) and pre-diluted controls/calibrator react for 30 minutes with *Saccharomyces cerevisiae* immobilised in microtiter wells. After washing away unbound serum components, antibodies specifically bound to antigen, if present in sample, are detected using enzyme labelled anti-human IgG. After 30 minutes incubation, unbound conjugate is removed by washing, and chromogenic substrate is added for 30 minutes. A colour develops if specific antibodies are present. Subsequently, a stop solution is added to terminate the enzymatic reaction and, thereafter, absorbance of the calibrator, controls and samples can be evaluated spectrophotometrically. Optical density (OD) is directly proportional to the amount of bound antibody. Arbitrary units are calculated based on sample OD and calibrator OD. Samples are considered positive for the presence of gASCA IgG antibodies if the sample units' value is greater than the cut-off value.

## KIT CONTENTS

S301100	gASCA Microwell Plate 96 well	<b>1 Plate</b> in sealed pouch with desiccant
G300023	IBDX™ Sample Diluent	<b>25 mL</b> clear solution in

		60 mL white bottle
G300022	Wash Concentrate 20x Sufficient for 1000mL	<b>50 mL</b> clear solution in 60 mL white bottle
S301025	<i>g</i> ASCA HRP IgG Conjugate horseradish peroxidase	<b>14 mL</b> red solution in 15 mL white bottle
G300024	TMB Chromogen 3,3',5,5'-Tetramethylbenzidine	<b>14 mL</b> clear solution in 15 mL amber bottle
G300021	HRP Stop Solution	<b>14 mL</b> clear solution in 15 mL white bottle
S301140	<i>g</i> ASCA IgG Calibrator	<b>1 mL</b> clear solution in 1 mL blue capped tube
S301130	<i>g</i> ASCA IgG Negative Control	<b>1 mL</b> clear solution in 1 mL blue capped tube
S301120	<i>g</i> ASCA IgG Positive Control	<b>1 mL</b> clear solution in 1 mL blue capped tube
SL701300	Instructions for use	<b>1 IFU</b>

## OTHER REQUIRED MATERIALS

10 mm X 60 mm tubes for dilution; 10 µL, 100 µL, and 1000 µL pipettes, with appropriate disposable pipette tips; 1 L container for diluted Wash Concentrate; microplate reader with 450 nm filter; General laboratory apparatus; Distilled or deionized water.

## STORAGE AND STABILITY

On arrival, store the kit at 2 - 8°C. Reagents remain stable until the expiration date when stored and handled as indicated. Follow good laboratory practices to minimize microbial and cross contamination of reagents once opened. It is important to protect the unused wells from excess moisture. Do not use kits beyond their expiration date.

Diluted Wash Concentrate is stable for 21 Days at 2 - 8°C. Exposure to 50°C or more for more than few hours will destroy the kit. Do not freeze.

## PATIENT SAMPLES

### Specimen collection and storage

Patient samples, serum should be collected by venipuncture as prescribed by local regulatory requirements. Following collection, the serum should be separated immediately from red blood cells. NCCLS Document H18-A recommends the following storage conditions for samples: 1) Store samples at room temperature for no longer than 8 hours. 2) Refrigerate the sample at 2 - 8°C if the assay will not be completed within 8 hours. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing. Repeated freezing and thawing may affect results. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

## PROCEDURAL NOTES

Allow samples and components to reach room temperature prior to the assay. Take care to agitate samples gently in order to ensure homogeneity and mix the components well prior to use in the assay

Dilution should be performed immediately before use.

Do not use kit components beyond their expiration date.

Do not interchange or mix kit components from different lots.

Have all reagents and samples ready before start of the assay. Once started, the assay should be performed without interruption and incubations should be accurately timed to achieve reliable and consistent results.

Perform the assay steps in the order indicated.

The assay calibrator and controls are manufactured from diluted human serum. Although these materials have been tested for presence of biohazardous agents, proper precautions should be taken while handling. Treat all human source material as potentially infectious. Normal clinical laboratory safety procedures should be maintained at all times. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.

### Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of automated equipment.

### Manual washing:

Discard liquid from wells by inverting the plate. Vigorously tap the microwell frame with wells downside on clean absorbent paper. Pipette 300 µL of diluted Wash Concentrate into each well, wait for 20 seconds. Repeat the whole procedure three more times.

### TMB Chromogen:

Avoid exposure of the TMB chromogen solution to light!

## PREPARATION

### Dilute wash concentrate:

Dilute the Wash Concentrate 1:20 with distilled water (e.g., 50 mL plus 950 mL). Prepare 50 mL of diluted Wash Concentrate (1x) per 8 wells or 600 mL for 96 wells.

### Samples:

Dilute serum samples 1:101 with IBDX™ Sample Diluent (5 µL serum+ 500 µL sample diluent). Mix well! Dilution should be performed immediately before use. **It is recommended that samples be run in duplicate.**

### Microwell plate:

The microwell plate is sealed in a foil pouch with desiccant. The plate consists of a frame and 12 (8 well) strips. Allow the sealed microwell plate to reach room temperature before opening. Calculate the number of wells required for the assay. Remove unused strips from the frame, replace and store in the foil pouch, together with desiccant, seal tightly and store at 2 - 8°C.

## ASSAY PROCEDURE

**ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY.**

### Print the Assay Scheme – last page

- Prepare** reagents and samples. Dilute patients' samples (1:101) in IBDX Sample Diluent.
- Add 50 µL** each of the *g*ASCA IgG Calibrator, the *g*ASCA IgG Positive Control, the *g*ASCA IgG Negative Control and the diluted (1:101) patient samples to different wells.
- Cover the wells and **incubate** for **30 minutes** at room temperature on a level surface.
- Wash** step: Thoroughly aspirate the contents of each well. Add to all wells 300 µL of the diluted Wash Concentrate; then aspirate. Repeat this cycle three more times for a total of four washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well.

Maintain the same procedural sequence for the aspiration as was used for the sample addition.

5. **Add 100 µL** of the gASCA HRP IgG Conjugate to each well. Conjugate should be pipetted using standard aseptic conditions and good laboratory techniques. Remove only the amount necessary for the assay. TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.
6. **Incubate** wells for **30 minutes** as described in step 3.
7. **Wash** step. Repeat step 4.
8. **Add 100 µL** of TMB Chromogen to each well.
9. **Incubate** for **30 minutes** at room temperature (keep away from direct light).
10. **Add 100 µL** of HRP Stop Solution to each well. Proceed following the same procedural sequence and timing as was used for addition of the TMB Chromogen.
11. Gently tap plate to mix wells. Thereafter **incubate** for **2 – 5 minutes** at room temperature on a level surface.
12. **Read** the absorbance (OD) of each well at 450 nm within one half hour of stopping the reaction.

## DATA PROCESSING

The result for each sample is calculated by dividing the average OD of the sample by the average OD of the gASCA IgG Calibrator, multiplied by the number of units denoted on the gASCA IgG Calibrator tube label.

$$\text{Sample Value (units)} = \frac{\text{Average Sample OD}}{\text{gASCA IgG Calibrator OD}} \times \text{gASCA IgG Calibrator (units)}$$

The result correlates with the quantity of antibody present in the patient sample in a non-linear fashion.

Please note that the calibrator units ARE NOT the cut-off values. The cut-off values appear in the Interpretation of results section.

## QUALITY CONTROL

The gASCA IgG Calibrator, gASCA IgG Positive Control and Negative Control should be included in every run to verify assay integrity and accuracy. The gASCA IgG Calibrator, gASCA IgG Positive Control and gASCA IgG Negative Control are prediluted, and therefore ready for use. They do not function as a control for procedural methods associated with specimen dilution.

Assay results are considered valid when the following criteria are met: A) The absorbance of the gASCA IgG Positive Control must be higher than the absorbance of the gASCA IgG Calibrator, which must be higher than the absorbance of the gASCA IgG Negative Control. B) The gASCA IgG Positive Control must have an absorbance **higher than 1.2**, whereas the gASCA IgG Negative Control absorbance should be **below 0.4**.

If any of the above criteria are not met, the assay should be considered invalid and therefore repeated.

## KIT PERFORMANCE

**Calibration** – Due to lack of international reference material the gASCA IgG calibrator is denoted in arbitrary units.

**Analytical sensitivity** - The analytical sensitivity of the gASCA IgG is less than 10 units.

**Precision**

Within Assay Imprecision < 11%  
Between Assay Imprecision < 15%

## INTERPRETATION OF RESULTS

### Differentiation between Crohn's Disease, Ulcerative Colitis, and non-IBD

For each sample, units for gASCA, ALCA, AMCA and ACCA are calculated as explained in the package insert for the individual assay. Use the following cut-off values table to determine whether the patient is positive or negative for the individual IBDX markers. These cut-off values were established on the basis of several studies using the IBDX panel on different cohorts. However, since the ELISA assay is a very sensitive technique, capable of detecting even small differences in a patients' population, the values shown below are suggested values only. Each laboratory should establish its own cut off values.

IBDX Cut-off values table:

Result	Cut-off values for			
	gASCA	ALCA	ACCA	AMCA
<b>Negative</b>	<45	<55	<80	<90
<b>Equivocal *</b>	45-50	55-60	80-90	90-100
<b>Positive</b>	>50	>60	>90	>100

\* Repeating the sample assay is recommended

Results should then be reported as follows:

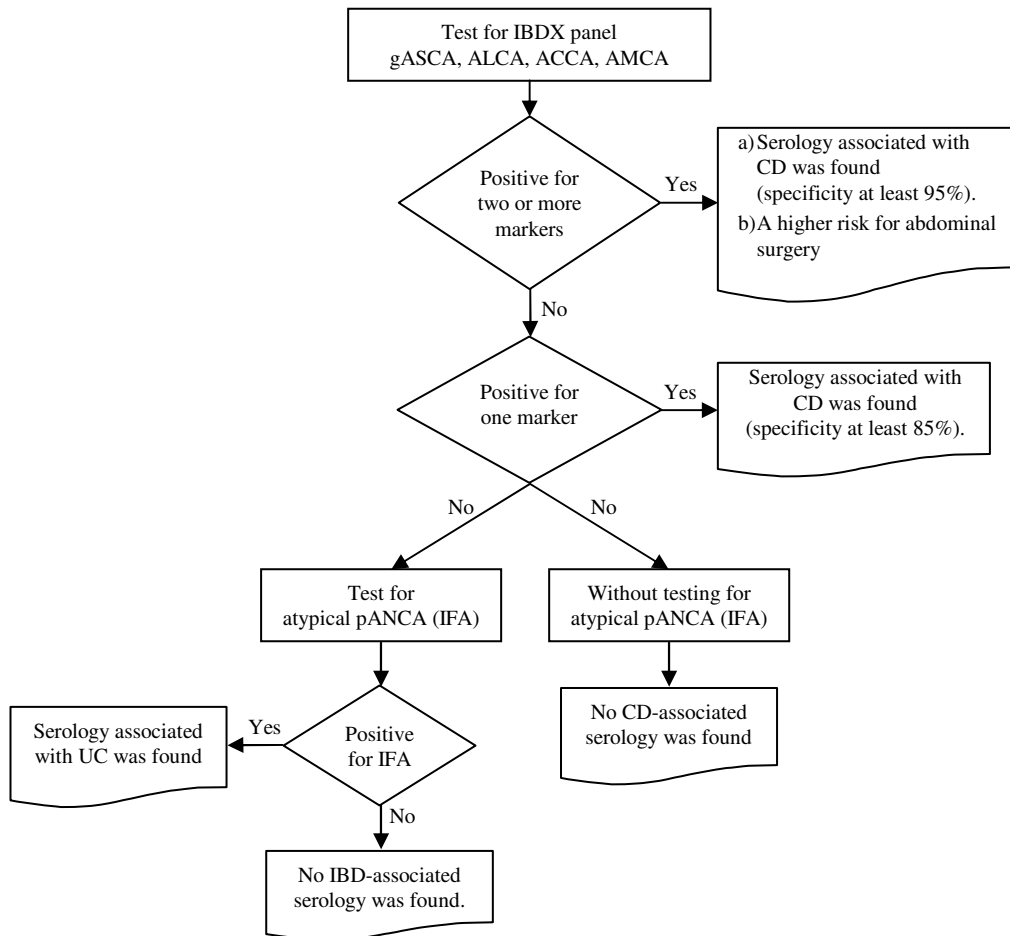
- i) If the patient is positive for *one* of the markers, report that serology associated with CD was found (specificity at least 85%).
- ii) If the patient is positive for *two or more* of the markers, report as follows:
  - a) Serology associated with CD was found (specificity at least 95%).
  - b) A higher risk for abdominal surgery relative risk of 1.7 when compared to a patient negative for all 4 markers (Ferrante, M., et al., 2006).
- iii) If the patient is negative for all markers, report that no serology associated with CD was found.

In order to exclude the possibility of UC, it is recommended to further test IBDX panel negative samples for atypical pANCA (IFA).

If patient is found *positive* for IFA and *negative* for IBDX panel, report that serology associated with UC was found.

If patient is found *negative* both for IFA and for the IBDX panel, report that no IBD-associated serology was found.

The following flow chart summarizes panel interpretation:



**PERFORMANCE**

**Differentiation between Crohn’s Disease, Ulcerative Colitis and non-IBD**

**Study I**

Sera of 247 German patients (149 CD, 50 UC and 48 non-IBD healthy controls) were tested retrospectively in a blinded fashion for the presence of IBDX markers and for atypical pANCA. IBD patients’ diagnosis was based on clinical, endoscopic, histological and radiological criteria.

The following tables outline the diagnostic performance for each assay independently:

CD vs. UC:

Interpretation	ACCA	ALCA	AMCA	gASCA	pANCA*
Sensitivity (%)	10.7	23.5	13.4	60.4	42.9
Specificity (%)	94.0	98.0	96.0	88.0	92.1
Positive predictive value (%)	84.2	97.2	90.9	93.8	63.6
Negative predictive value (%)	26.1	30.1	27.1	42.7	83.3
Efficiency (%)	31.7	42.2	34.2	67.3	80.1

\* Atypical pANCA (IFA) performance for differentiation between UC and CD.

CD vs. non-CD:

Interpretation	ACCA	ALCA	AMCA	gASCA	pANCA*
Sensitivity (%)	10.7	23.5	13.4	60.4	42.9
Specificity (%)	96.1	95.1	97.1	90.3	94.0
Positive predictive value (%)	80.0	87.5	87.0	90.0	63.6
Negative predictive value (%)	42.7	46.2	43.7	61.2	87.0
Efficiency (%)	45.6	52.8	47.6	72.6	83.9

\* Atypical pANCA (IFA) performance for differentiation between UC and all other non-UC.

The following table outlines the diagnostic performance based on the combined use of gASCA, ALCA, ACCA, and AMCA as a panel without pANCA.

CD vs. non-CD:

Interpretation	At least one positive	At least two positive	At least three positive
Sensitivity (%)	69.1	30.9	8.1
Specificity (%)	82.0	96.0	98.1
Positive predictive value (%)	92.1	95.8	92.3
Negative predictive value (%)	47.1	31.8	26.3
Efficiency (%)	72.4	47.2	30.7

The following table outlines the diagnostic performance based on combined use of gASCA, ALCA, ACCA, AMCA as a panel together with pANCA.

CD vs. UC vs. non-IBD:

Interpretation	CD	UC	Non
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	At least 2 positive markers	One positive marker		IBD
Sensitivity (%)	30.9	55.3	34.7	83.3
Specificity (%)	98.1	83.2	97.0	67.7
Positive predictive value (%)	95.8	77.0	73.9	38.1
Negative predictive value (%)	49.5	64.6	85.8	94.4
Efficiency (%)	58.3	69.1	84.7	70.7

### Study II\*

The sera of 1498 Belgium patients (913 CD, 272 UC, 113 diverticular disease, and 200 healthy controls) were tested retrospectively for the presence of IBDX markers (gASCA, ALCA, and AMCA). IBD patients' diagnosis was based on clinical, endoscopic, histological and radiological criteria.

\* ACCA IgA was not included in this study

The following tables outline the diagnostic performance based on combined use of gASCA, ALCA and AMCA without pANCA.

#### CD vs. UC:

Interpretation	At least one positive	At least two positive	At least three positive
Sensitivity (%)	66.0	29.0	7.9
Specificity (%)	74.6	94.6	99.6
Positive predictive value (%)	89.7	95.0	98.6
Negative predictive value (%)	39.6	28.5	24.4
Efficiency (%)	68.0	44.1	28.9

#### CD vs. non-CD:

Interpretation	At least one positive	At least two positive	At least three positive
Sensitivity (%)	66.0	29.0	7.9
Specificity (%)	92.3	100.0	100.0
Positive predictive value (%)	96.2	100.0	100.0
Negative predictive value (%)	48.2	32.6	27.1
Efficiency (%)	72.8	47.1	31.4

The following table outlines the diagnostic performance based on combined use of gASCA, ALCA and AMCA together with pANCA.

Interpretation	CD		UC	Non IBD
	At least 2 positive markers	One positive marker		
Sensitivity (%)	29.0	52.2	36.5	96.5
Specificity (%)	96.8	85.3	89.9	23.6
Positive predictive value (%)	93.0	79.2	54.0	40.3
Negative predictive value (%)	48.3	62.5	81.2	92.7
Efficiency (%)	56.6	68.2	76.7	49.0

### Study III (Dotan, I., et al., 2006)

The sera of 331 Israeli patients (124 CD, 106 UC, 27 celiac disease, 20 irritable bowel syndrome, 14 other gastro diseases, and 40 healthy controls) were tested retrospectively for the presence IBDX markers (gASCA, ALCA, and ACCA) in a blinded fashion. IBD patients' diagnosis was based on clinical, endoscopic, histological and radiological criteria.

#### CD vs. UC:

Interpretation	At least one positive	At least two positive	At least three positive
Sensitivity (%)	77.4	26.6	3.2
Specificity (%)	90.6	99.1	100.0
Positive predictive value (%)	90.6	97.1	100.0
Negative predictive value (%)	77.4	53.6	46.9
Efficiency (%)	83.5	60.0	47.8

#### CD vs. non-IBD:

Interpretation	At least one positive	At least two positive	At least three positive
Sensitivity (%)	77.4	36.3	8.1
Specificity (%)	70.3	96.0	99.0
Positive predictive value (%)	76.2	91.8	90.9
Negative predictive value (%)	71.7	55.1	46.7
Efficiency (%)	74.2	63.1	48.9

## Risk of CD patients for abdominal surgery

The association between being positive for multiple markers and having an abdominal surgery or complicated disease (fistula or stricture) was tested in two independent cohorts of CD patients: a Belgium cohort (n=745) (Ferrante, M., et al., 2006), and a German cohort (n=148). The following table summarizes the results.

#### Risk for CD related abdominal surgery אֲרֻטָּוּת

Number of positive markers	Belgium cohort (CD n=745) Need surgery/total (%), Odds ratio (95% CI)	German cohort (CD n=148) Need surgery/total (%), Odds ratio (95% CI)
0	83/207 (40%)	23/46 (50%)
1	147/270 (54%)*, OR 1.8 (1.2-2.5)	52/57 (91%)*, OR 10.4 (3.5-30.7)
2 or more	192/278 (69%)*, OR 3.3(2.3-4.8), RR 1.74	39/46 (84%)*, OR 5.5(2.0-15.0), RR 1.68

Markers included in analysis are gASCA, ALCA, ACCA, and AMCA.

\*p<0.05 chi square versus group of negative for all markers.

OR – Odds ratio versus group of negative for all markers. RR – relative risk calculated for CD patients positive for two or more of the markers vs CD patients negative for all 4 markers.

## REFERENCES

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## ASSAY SCHEME

	Action	Material	Description
1	<b>Preparation</b>	All reagents Patient samples	Bring to room temperature (20 - 26°C) Dilute (1:101) in IBDX Sample Diluent
2	<b>Add</b>	Pos. / Neg. Controls	50 µL each
		Calibrator	50 µL
		Diluted samples	50 µL each
3	<b>Incubate</b>		30 minutes at room temperature (20 - 26°C)
4	<b>Wash</b>		Wash with 4x 300 µL diluted Wash Concentrate (x1)
5	<b>Add</b>	HRP Conjugate	100 µL all wells
6	<b>Incubate</b>		30 minutes at room temperature (20 - 26°C)
7	<b>Wash</b>		Wash with 4x 300µL diluted Wash Concentrate (x1)
8	<b>Add</b>	TMB Chromogen	100 µL all wells
9	<b>Incubate</b>		30 minutes at room temperature (20 - 26°C). Protect from light!
10	<b>Add</b>	HRP Stop Solution	100 µL all wells
11	<b>Incubate</b>		Mix gently by tapping then let stand 2-5 minutes at room temperature (20 - 26°C)
12	<b>Read</b>		Read OD at 450 nm

## SAFETY PRECAUTIONS

- **This kit is for *in vitro* use only.** Glycominds Ltd. and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The assay should be performed by trained technical staff only.
- Some of the reagents contain small amounts of sodium azide (< 0.09 % w/v), and proclin (0.05 % v/v) as preservatives. They must not be swallowed or allowed to come into contact with eyes or skin.
- One kit component – Stop Solution – contain a hazardous reagent in an amount that requires labelling. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice; after contact with skin, wash immediately with plenty of soap-suds. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
- 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 complete 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 and coats. Never pipette material by mouth. Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- Avoid release into the environment. Observe local environmental regulations when disposing of wastes.

## TROUBLESHOOTING

Problem:	Possible causes:	Solution:
<i>Control values out of range</i>	1. Incorrect temperature, timing, improper pipetting, reagents not mixed.	1. Check that the time and temperature were correct. Pipette carefully. Repeat assay
	2. Optical pathway not clean.	2. Check for dirt or air bubbles. Wipe bottom of plate and reread.
<i>All results negative</i>	One or more reagents not added or added in wrong sequence.	Recheck procedure. Check for unused reagent. Repeat assay.
<i>All assay results yellow</i>	1. Contaminated buffers or reagents.	1. Check all solutions for turbidity.
	2. Washing solution contaminated.	2. Use clean container. Check quality of water.
	3. Improper dilution of sample.	3. Dilute sample appropriately. Repeat assay.
<i>Poor precision</i>	1. Pipette delivery CV greater than 5%.	1. Check calibration of pipette.
	2. Samples or reagents not mixed sufficiently or not equilibrated to room temperature.	2. Mix all reagents gently but thoroughly and equilibrate to room temperature.
	3. Reagent addition taking too long and inconsistent time intervals.	3. Develop consistent uniform technique and/or use automated equipment.
	4. Washing not consistent; trapped bubbles; washing solution left in the wells.	4. Check that all wells are filled and aspirated uniformly. Dispense liquid above level of reagent in well. After the last wash, empty the wells by tapping on an absorbent tissue.