Instructions for use

IBDX® *anti-L* IgA ELISA Kit

Semi-quantitative ELISA for anti-*L* IgA antibodies in human serum

96 determinations

For research use only

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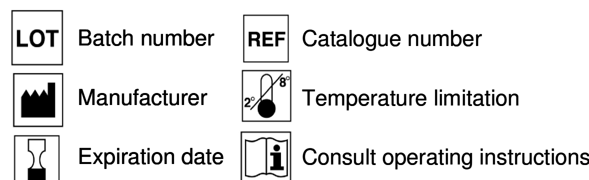

INTENDED USE

The IBDX *anti-L* IgA ELISA kit is an indirect solid phase enzyme-linked immunosorbent assay (ELISA) for the detection of circulating IgA class antibodies against laminarin antigen in human serum.

PRINCIPLES OF THE PROCEDURE

Diluted serum samples (1:101) and pre-diluted controls/calibrator react for 30 minutes with laminarin 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 IgA. 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 *anti-L* IgA antibodies if the sample units' value is greater than the cut-off value.

KIT CONTENTS

AL307100	*Anti-L* Microwell Plate 96 well	**1 Plate** in sealed pouch with desiccant
G300023	Sample Diluent	**25 mL** yellow solution in 60 mL white bottle
G300022	Wash Concentrate 20x Sufficient for 1000mL	**50 mL** clear solution in 60 mL white bottle
AL307025	*Anti-L* HRP IgA Conjugate horseradish peroxidase	**14 mL** red solution in 15 mL white bottle
G300024	TMB Chromogen 3,3',5,5'-Tetramethylbenzidine	**14 mL** clear solution in 15 mL amber bottle
G300021	HRP Stop Solution	**14 mL** clear solution in 15 mL white bottle
AL307140	*Anti-L* IgA Calibrator	**1 mL** clear solution in 1 mL orange capped tube
AL307130	*Anti-L* IgA Negative Control	**1 mL** clear solution in 1 mL orange capped tube
AL307120	*Anti-L* IgA Positive Control	**1 mL** clear solution in 1 mL orange capped tube
ALL707300	Instructions for use	**1 IFU**

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, storage, and transport

The patient's serum sample, should be collected by venipuncture as prescribed by local regulatory requirements. Following collection, the serum should be separated immediately from clot. Blood collection tubes with clot activator and separation gel can be used. After separation from blood clot sera samples should be refrigerate at 4°C (2 - 8°C) if the assay will not be started within 8 hours. Sera samples can be transported at 4°C (2 - 8°C). Sera samples are stable at 4°C (2 - 8°C) for up to 14 days. For longer transport time or longer storage time the samples should be

frozen at -20℃ or lower. Samples can not shipped under ambient temperature.

Repeated freezing and thawing may affect results and should be avoided.

Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. Addition of azide or other preservatives to the test samples may adversely affect the results. Heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed, lipemic serum, or bacterially contaminated 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 or plasma 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 and calibrator be run in duplicates.**

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℃) PRIOR TO BEGINNING THE ASSAY.

Print the Assay Scheme – last page

1. **Prepare** reagents and samples. Dilute patients' samples (1:101) in IBDX Sample Diluent.
2. **Add 50 µL** each of the *anti-L* IgA Calibrator, the *anti-L* IgA Positive Control, the *anti-L* IgA Negative Control and the diluted (1:101) patient samples to different wells.
3. Cover the wells and **incubate** for **30 minutes** at room temperature on a level surface.
4. **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 *anti-L* HRP IgA 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 anti-L IgA Calibrator, multiplied by the number of units denoted on the anti-L IgA Calibrator tube label.

$$\text{Sample Value (units)} = \frac{\text{Average Sample OD}}{\textit{anti-L}\text{ IgA Calibrator OD}} \times \textit{anti-L}\text{ IgA Calibrator (units)}$$

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 *anti-L* IgA Calibrator, *anti-L* IgA Positive Control and Negative Control should be included in every run to verify assay integrity and accuracy. The *anti-L* IgA Calibrator, *anti-L* IgA Positive Control and *anti-L* IgA 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 *anti-L* IgA Positive Control must be higher than the absorbance of the *anti-L* IgA Calibrator, which must be higher than the absorbance of the *anti-L* IgA Negative Control. B) The *anti-L* IgA Positive Control must have an absorbance **higher than 1.2**, whereas the *anti-L* IgA 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

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

INTERPRETATION OF RESULTS

For each sample, units for *anti-L* IgA, are calculated as explained in the package insert for the individual assay. Use the following cut-off values table to determinate 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 very sensitive to technique used, the values shown below are suggested values only. Each laboratory should establish its own cut off values.

anti-L Cut-off values table:

Result	***Anti-L* IgA**
Negative	<45
Equivocal *	45-50
Positive	>50

* Repeating the sample assay is recommended.

ASSAY SCHEME

	Action	Material	Description
1	**Preparation**	All reagents	Bring to room temperature (20 - 26ºC)
		Patient samples	Dilute (1:101) in IBDX Sample Diluent (5μL sera in 500μL Diluent)
2	**Add**	Pos. / Neg. Controls	50 μL each
		Calibrator	50 μL
		Diluted samples	50 μL each
3	**Incubate**		30 minutes at room temperature (20 - 26ºC)
4	**Wash**		Wash with 4x 300 μL diluted Wash Concentrate (x1)
5	**Add**	HRP Conjugate	100 μL all wells
6	**Incubate**		30 minutes at room temperature (20 - 26ºC)
7	**Wash**		Wash with 4x 300μL diluted Wash Concentrate (x1)
8	**Add**	TMB Chromogen	100 μL all wells
9	**Incubate**		30 minutes at room temperature (20 - 26ºC). Protect from light!
10	**Add**	HRP Stop Solution	100 μL all wells
11	**Incubate**		Mix gently by tapping then let stand 2-5 minutes at room temperature (20 - 26ºC)
12	**Read**		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 components – 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:**
Control values out of range	**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.
All results negative	One or more reagents not added or added in wrong sequence.	Recheck procedure. Check for unused reagent. Repeat assay.
All assay results yellow	**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.
Poor precision	**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.