EKVITESTLAB LLC

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STATEMENT

We, EKVITESTLAB LLC, having a registered office at Velyka Vasylkivska street 114, Kyiv, 03150, Ukraine assign SRL SANMEDICO having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova, as authorized representative in correspondence with the conditions of directive 98/79/EEC.

We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova.

Date: 03 January 2025

Signature: _____

Director, Anna Yurchuk

Digitally signed by Dolgopol Iulian
Date: 2025.03.12 11:54:21 EET
Reason: MoldSign Signature
Location: Moldova
MOLDOVA EUROPEANĂ

УІС УКРАЇНСЬКИЙ ІНСТИТУТ СТАНДАРТІВ

Сертифікат
Certificate

№ Q1M 804 255 C1

Система управління якістю виробника:
Quality management system of manufacturer

Товариство з обмеженою відповідальністю «ЕКВІТЕСТЛАБ»
«EKVITESTLAB» Limited Liability Company

Місцезнаходження юридичної особи: вул. Велика Васильківська 114, м. Київ, 03150, Україна
Location of the legal entity: 114 Velyka Vasylkivska St., Kyiv, 03150, Ukraine
Фактичне місцезнаходження: Україна, 03057, м. Київ, проспект Берестейський 60/2
Actual location: 60/2 Beresteysky Avenue, Kyiv, 03057, Ukraine

відповідає вимогам:
meets the requirements of

ДСТУ EN ISO 13485:2018
Вироби медичні. Системи управління якістю. Вимоги щодо регулювання (EN ISO 13485:2016, IDT; ISO 13485:2016, IDT
Medical devices – Quality management systems – Requirements for regulatory purposes)

Сфера застосування:
Scope
Проектування, розробка, виробництво, зберігання та реалізація ІФА-наборів для діагностики in vitro
Design, development, production, storage and sale of ELISA kits for in vitro diagnostics

Сертифікат виданий ТОВ «Український Інститут Стандартів», місцезнаходження: будинок 1, вулиця Олександрівська, місто Київ, 03062, Україна.
Атестат акредитації НААУ від 30 червня 2020 року № 8О141.
Certificate is issued by LLC Ukrainian Standards Institution: building 1, Oleksandrivska street, Kyiv, 03062, Ukraine.
Accreditation certificate registered on June 30, 2020 No. 8O141

Рішення №: **255-000**
Decision No.:

Дата видачі: **01.04.2024**
Issue date:

Дійсний з: **01.04.2024**
Effective date:

Дійсний до: **31.03.2027**
Expiry date:

Директор *Director*
Наталія СТЕПАНКІВСЬКА
Natalia STEPANKIVSKA

8О141
Сертифікація систем менеджменту

Сертифікат чинний за умови проведення щорічного наглядового аудиту. Чинність сертифікату необхідно перевірити на офіційному веб-сайті www.usi.biz.ua або за телефоном: +38-050- 818-7-333
Certificate is valid if the annual surveillance audit has been conducted. The validity of the certificate shall be checked on the official website www.usi.biz.ua or by tel.: +38-050- 818-7-333

Declaration of Conformity

According to annex III of the Council Directive 98/79/EC on in vitro diagnostic medical device We,

EKVITESTLAB LLC

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Declare under our sole responsibility that the following in vitro diagnostic medical devices other than those covered by annex II and devices for performance evaluation

EQUI Ascaris lumbricoides IgG - ELISA kit for the qualitative detection of IgG antibodies to *Ascaris lumbricoides*, REF EI-601

Meet the provisions of the Council Directive 98/79/EC concerning medical devices which apply to them.

Undersigned declares to fulfill the obligations imposed by Annex III section 2 to 5:

- availability of the technical documentation set in Annex III (section 3), allowing the assessment of conformity of the product with the requirements of the Directive.
- the manufacturer shall take necessary measures to ensure that the manufacturing process follows the principles of quality assurance as appropriate for the products manufactured (Annex III section 4).
- the manufacturer shall institute and keep up to date a systematic procedure to review experience gained from devices in the post-production phase and to implement appropriate means to apply any necessary corrective actions (Annex III section 5).

Conformity assessment was performed according to Article 9 (7) and Annex III, section 3.

Our current Quality System is formatted to international standards:

- **ISO 13485:2016 «Medical devices — Quality management systems — Requirements for regulatory purposes»**

Corporate Contact Information

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RESPONSIBLE PERSON'S name: Anna Yurchuk
Position: Director

SIGNATURE : 

Date : October 25, 2021
Stamp

European Authorized Representative:
Registered Address:
Obelis s.a.
Bd. Général Wahis 53
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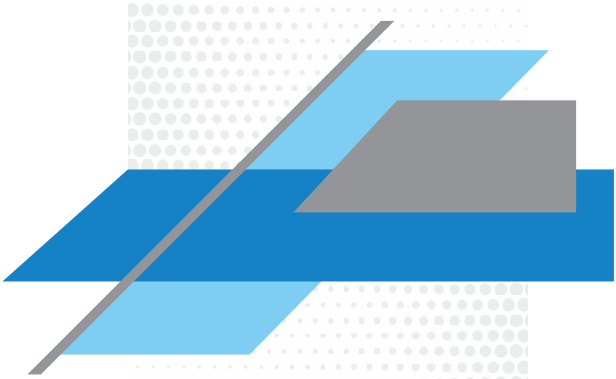
Representative: Mr. Gideon ELKAYAM (CEO)

Ascaris lumbricoides IgG

ELISA kit for the qualitative detection of IgG antibodies to *Ascaris lumbricoides*

Instructions for use



IVD

REF EI-601

Σ 96 tests

CE

EQUI Ascaris lumbricoides IgG

ELISA kit for the qualitative detection of
IgG antibodies to *Ascaris lumbricoides*

1. INTENDED USE

The «EQUI Ascaris lumbricoides IgG» is ELISA kit intended to qualitatively detect anti-*Ascaris lumbricoides* IgG in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) in order to diagnose lumbricosis. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

Target group: children, rural people, summer visitors.

Usage: ELISA kit is used in clinical diagnostic laboratories and other institutions engaged in *in vitro* diagnostics.

2. CLINICAL SIGNIFICANCE

Ascaris lumbricoides is a human parasite resulting in lumbricosis — one of the most common helminthiases in the world. By some estimates, over a milliard of people infested with acaricides are on earth.

Human ascaris belongs to *Nematoda* roundworms infesting the small intestine of a man who is its exclusive host. *Ascaris lumbricoides* eggs are excreted in the environment with faeces of the infested man. In a warm, wet soil, ascaris larvae develops in the eggs, therefore eggs become invasive only after a maturation period (2 to 3 weeks at 25–30 °C, lower temperatures require longer term). After infestation, larvae leave eggs in the human intestine, penetrates blood circulation and migrate to the liver and lungs with blood flow. The larvae move to the pharynx from the lungs, and here they are re-ingested and further enter the small intestine. In 2 to 3 months, adult ascaris able to propagate develops from larvae in the small intestine.

The helminths are transferred by faecal-oral route upon injection of mature eggs of *Ascaris lumbricoides* with soil-contaminated vegetables, fruits, water, as well as through dirty hands after contact with soil. Lumbricosis is conditionally divided into the early stage (migration of larvae) and late stage (parasitism of adults in the intestine). Invasion is asymptomatic in most cases. Primary feeling of being unwell occurs as early as several days after infestation and is accompanied by weakness, abdominal pain, nausea. Migration of larvae to the lungs may manifest as rales and cough. In some cases, intense invasion may result in pneumonia and liver damage. However, the most common symptom of early lumbricosis are allergic reactions due to hypersensitivity to metabolic products of larvae.

Late stage manifests as decreases appetite, abdominal pain, vomiting, diarrhoea, constipation. Massive ascaris invasion may result in the intestinal obstruction with a lump of helminths or rupture of the walls with peritonitis. When ascarides penetrate other organs, complications may develop such as

hepatitis, cholangitis, pancreatitis and even asphyxia. Cases of neurological disorders sometimes develop in lumbricosis, namely: headache, irritability, sleep impairment, inattention, etc. If no timely treatment is started for intense invasion, it may lead to death, especially in younger children.

Strong immune response to *Ascaris lumbricoides* invasion develops as early as at the early stage. It includes cellular and humoral immunity. Antigens of ascaris larvae stimulate secretion of all-class specific immunoglobulins, however, the level of specific and total IgE antibodies is the highest. The intensity of the immune response (including increased IgG titres) correlates with the massiveness of the invasion.

For diagnosis of lumbricosis, parasitologic stool test for presence of ascaris larvae and eggs is the most common. X-ray imaging of the lungs is additionally applied at the early stage of invasion. Complete blood count (eosinophilia develops in lumbricosis) and detection of serum anti-*Ascaris lumbricoides* antibodies also is included in the set of exams. The presence of specific anti-ascaris antibodies may suggest asymptomatic invasion, and allows initiation of treatment before complications develop in conjunction with other diagnostic instruments.

3. ANALYSIS PRINCIPLE

The procedure of testing for anti-*Ascaris lumbricoides* IgG in «EQUI Ascaris lumbricoides IgG» ELISA kit is based on «indirect» solid-phase ELISA with a two-stage incubation. Antigens of *Ascaris lumbricoides* larvae are entrapped in the wells. During the first step of incubation of ELISA plate wells with test samples, specific anti-*Ascaris lumbricoides* antibodies (if present in the samples) bind to the solid-phase antigens. The wells are washed to remove unbound antibodies and have only specific antigen-antibody complexes left. Then, a conjugate of anti-species IgG monoclonal antibodies with horseradish peroxidase is added, which binds to solid-phase immune complexes. Unbound components are removed by washing. Antigen-antibody complexes are detected by adding a solution of chromogen 3,3',5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After 30-minute incubation, the reaction is stopped by adding the stop solution. The optical density (OD) in the wells is determined using a spectrophotometer at 450/620-695 nm. The intensity of the yellow colour is proportional to the level of antibodies in the sample.

4. MATERIALS AND EQUIPMENT

4.1. Contents of the ELISA kit

STRIPS	1 x 96 wells	**Microplate** Each plate well is coated with *Ascaris lumbricoides* antigen. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months

CONTROL +	1 x 0,25 ml	**Positive control** Conjugated specific monoclonal antibody solution with preservative (pink). Store at 2-8 °C
CONTROL -	1 x 0,6 ml	**Negative control** Negative human serum with a preservative (yellow). Store at 2-8 °C
DIL SAMPLE	1 x 13 ml	**Serum dilution solution** Buffer solution with a milk extract, a detergent and a preservative (brown). Store at 2-8 °C
SOLN CONJ	1 x 13 ml	**Conjugate solution (ready to use)** Buffer solution of monoclonal antibodies to human IgG, conjugated with horseradish peroxidase, with stabilizers and preservative (green). Store at 2-8 °C
SOLN TMB	1 x 13 ml	**TMB solution (ready to use)** TMB solution, H_2O_2, a stabilizer, a preservative (colourless). Store at 2-8 °C
TWEEN WASH 20x	1 x 50 ml	**Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days
SOLN STOP	1 x 13 ml	**Stop Solution (ready to use)** 0.5 mol H_2SO_4 solution (colourless). Store at 2-8 °C

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

5. PRECAUTIONS AND SAFETY

5.1. Precautions

Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI Ascaris lumbricoides IgG» ELISA kit;
– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;

– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;
– SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for SOLN CONJ and SOLN TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

5.2. Safety requirements

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI Ascaris lumbricoides IgG» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN TMB, SOLN STOP and SOLN CONJ with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

5.3. Waste inactivation and disposal

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;

– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

8. REAGENT PREPARATION

NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!

8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for 30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Add 100 µL of SOLN CONJ into each well. Cover the strips with a new piece of adhesive film and incubate for **30 minutes at 37 °C**.

9.9. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.10. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.11. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.12. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding, the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.13. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added

in blank well).

10. CALCULATION AND INTERPRETATION OF RESULTS

10.1. Calculation of results

Calculate the average OD for the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index (IP_{sample}).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$$IP_{sample} = OD_{sample}/CO, \text{ where } OD_{sample} \text{ is the OD sample.}$$

10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

CONTROL + OD ≥ 1,0

CONTROL - OD ≤ 0,150

CONTROL - $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ where Ncn is the OD for each Nc run

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

10.3. Interpretation of results

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE*
$IP_{sample} < 0,9$	NEGATIVE

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

11. PERFORMANCE CHARACTERISTICS

11.1. Analytical performance characteristics

Precision of measurement

Intra assay repeatability

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated in 24 replicates on one series of ELISA kits.

Sample No.	OD_{av}	IP_{av}	CV, %
547	0,504	1,43	2,9
671	0,753	2,13	3,6
413	1,165	3,30	3,1

Inter assay reproducibility

The coefficient of variation (CV) for three sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

Sample No.	OD_{av}	IP_{av}	CV, %
547	0,534	1,55	5,0
671	0,750	2,17	4,6
413	1,159	3,36	3,6

Analytical specificity

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 μmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

11.2. Diagnostic characteristics

To evaluate clinical sensitivity and specificity of «EQUI Ascaris lumbricoides IgG» ELISA kits, 55 serum samples from patients with clinical symptoms typical for lumbricosis and 60 serum samples from patients without clinical manifestations (seronegative in terms of *Ascaris lumbricoides*) were used. Clinical sensitivity of «EQUI Ascaris lumbricoides IgG» ELISA kits was 94.55 % and clinical specificity — 93.3 %.

Method characteristics in comparison with equal commercial ELISA kit was studied in target paediatric population (160 samples) and population of donors (346 samples). For paediatric population serum, relative specificity of «EQUI Ascaris lumbricoides IgG» ELISA kits was established at the level of 97.92 % and percent agreement was 95.51 %. For donor population serum, relative specificity of was 89.74 %, relative specificity — 96.30 % and percent agreement was 95.47 %.

12. LIMITATIONS OF ASSAY

Positive result in «EQUI Ascaris lumbricoides IgG» ELISA kit supports presence of anti-*Ascaris lumbricoides* specific IgG antibodies. Presence of this class antibodies in newborns is not an evidence of *Ascaris lumbricoides* invasion.

Inconclusive results may suggest a history of *Ascaris lumbricoides* invasion.

Negative result of «EQUI Ascaris lumbricoides IgG» ELISA kit supports the absence of anti- *Ascaris lumbricoides* IgG specific antibodies in the test sample or concentration of specific antibodies is below the sensitivity limit of the assay.

The results of serological test only are not the basis for final diagnosis. When establishing the diagnosis, the results of complex laboratory and instrumental tests, as well as clinical manifestations should be considered. Cross-reactions with antibodies to antigens of other helminths cannot be fully ruled out.

13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

Possible reasons	Solution
High background in all wells	
Contaminated washer	Clean the washer head and rinse according to the instructions for use
Poor quality or contaminated water	Use purified water with specific resistance ≥ 10 MΩ · cm
Use of poorly washed glassware	Use chemically clean utensils
Use of chlorinated disinfectants	Do not use chlorine disinfectants
Use of contaminated tips	Use new tips
Increased incubation times or change in the temperature conditions	Adhere to the incubation regime according to the instructions for use
High background in a row of wells	
Repeat application of TMB solution	TMB solution should be applied once
Contamination of the automatic pipette nozzle with conjugate solution	Clean the pipette and dial carefully liquid
Contamination of one of the washer's channel	Clean the flush channel, rinse washer
Received OD of the positive control is below the border value	
One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added	Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents
Reduced incubation times at any stage	Incubate according to instructions for use
The colour density of the wells fails to meet the obtained optical density value	
This may suggest that the optical beam has been displaced	Check the correct operation of the reader

14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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	Manufacturer
	Authorized Representative in the European Community
	In vitro diagnostic medical device
	Catalogue number
	Date of manufacture
	Use by date
	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Non-Sterile
	Consult instructions for use
	Keep away from sunlight
	Keep dry
	Compliance with EU safety requirements

Edition 8, 10.02.2022

For questions and suggestions regarding the ELISA kit contact:

Obelis s.a.
Bd Général Wahis 53
1030 Brussels
Belgium
Tel: +(32)2 732-59-54
Fax: +(32)2 732-60-03
mail@obelis.net

Ekvitestlab LLC
Velyka Vasylkivska St. 114, Kyiv, Ukraine, 03150
Tel: 0(800)31-89-87, +38 (044)334-89-87,
e-mail: info@equitest.com.ua, www.equitest.com.ua

ASSAY PROCEDURE SCHEME

Keep all reagents for 30 min at temperature18-25°C before use
Dispense 90 µl DIL SAMPLE into the wells (brown)
Add to 10 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from brown to blue)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN CONJ into all wells (green)
Cover strips with an adhesive film, incubate for **30 min at 37°C**
Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well)
Add 100 µl of SOLN TMB into all wells
Incubate for **30 min** in the dark at **18-25°C**
Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow)
Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm

CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

IP_{sample} - sample positivity index

INTERPRETATION OF RESULTS

$IP_{sample} > 1,1$	POSITIVE
$0,9 \leq IP_{sample} \leq 1,1$	BORDERLINE
$IP_{sample} < 0,9$	NEGATIVE

EC CERTIFICATE

Number: 2116030CE04

Full Quality Assurance System

Directive 98/79/EC on In Vitro Diagnostic Medical Devices, Annex IV excluding (4,6)
(List A, B and devices for self-testing)

Manufacturer:

General Biologicals Corporation

No. 6 Innovation First Road Hsinchu Science Park Baoshan Township, Taiwan, R.O.C.
30076 Hsinchu County 30076
Taiwan

For the product category(ies)

Enzyme immunoassays for the qualitative detection in human specimens of markers of HIV

DEKRA grants the right to use the EC Notified Body Identification Number illustrated below to accompany the CE Marking of Conformity on the products concerned conforming to the required Technical Documentation and corresponding batch release certificate(s) and meeting the provisions of the EC-Directive which apply to them:

0344

Documents, that form the basis of this certificate:

Certification Notice 2116030CN, initially dated 6 June 2008

DEKRA hereby declares that the above mentioned manufacturer fulfils the relevant provisions of 'Besluit in-vitro diagnostica', the Dutch transposition of the Council Directive 98/79/EC of October 27, 1998 concerning In vitro diagnostic medical devices, including all subsequent amendments. The manufacturer has implemented a quality assurance system for design, manufacture and final inspection for the above mentioned product category in accordance to the provisions of Annex IV of Council Directive 98/79/EC of October 27, 1998 and is subject to periodical surveillance. For placing on the market of List A devices an additional EC design examination certificate according to Annex IV (4) is mandatory.
The necessary information related to the quality assurance system of the manufacturer, including facilities and the reference to the relevant documentation, of the products concerned and the assessments performed, are stated in the Certification Notice which forms an integrative part of this certificate.

This certificate is valid until: 26 May 2025
Issued for the first time: 12 February 2020
Revised: 19 May 2022

DEKRA Certification B.V.

B.T.M. Holtus
Managing Director

J.A. van Vugt
Certification Manager

DEKRA Certification B.V. is Notified Body with ID no 0344

DEKRA Certification B.V. Meander 1051, 6825 MJ Arnhem P.O. Box 5185, 6802 ED Arnhem, The Netherlands
T +31 88 96 83000 F +31 88 96 83100 www.dekra.nl Company registration 09085396

EC DESIGN-EXAMINATION CERTIFICATE

Number: 2116030DE11

Directive 98/79/EC on In Vitro Diagnostic Medical Devices, Annex IV (4)
(List A)

Manufacturer:

General Biologicals Corporation

No. 6 Innovation First Road Hsinchu Science Park Baoshan Township, Taiwan, R.O.C. 30076 Hsinchu County 30076
Taiwan

For the product

GB HIV Ag-Ab COMB for qualitative screening in human serum or plasma (EDTA, Heparin and Citrate) of antibodies to HIV-1, HIV-2 and HIV-1 p24 antigen

Documents, that form the basis of this certificate:

Certification Notice 2116030CN, initially dated 6 June 2008
CE Marking of Conformity 2116030CE04
Addendum, initially dated 12 February 2020

DEKRA hereby declares that the design of the product(s) falling within the product category mentioned above, fulfils the relevant provisions of 'Besluit in-vitro diagnostica', the Dutch transposition of the Council Directive 98/79/EC of October 27, 1998 concerning In vitro diagnostic medical devices, including all subsequent amendments, based on an examination in accordance with Annex IV (4) of this Directive. The manufacturer has implemented a quality assurance system for the above mentioned product category in accordance to the provisions of Annex IV (4) of Council Directive 98/79/EC of October 27, 1998 and is subject to periodical surveillance.
The necessary information and the reference to the relevant documentation, of the products concerned and the examinations and assessments performed, are stated in the Certification Notice which forms an integrative part of this certificate.

This certificate is valid until: 26 May 2025
Issued for the first time: 12 February 2020
Revised: 19 May 2022

DEKRA Certification B.V.

B.T.M. Holtus
Managing Director

J.A. van Vugt
Certification Manager

DEKRA Certification B.V. is Notified Body with ID no 0344

DEKRA Certification B.V. Meander 1051, 6825 MJ Arnhem P.O. Box 5185, 6802 ED Arnhem, The Netherlands
T +31 88 96 83000 F +31 88 96 83100 www.dekra.nl Company registration 09085396

ADDENDUM

Belonging to certificate: 2116030DE11

EC DESIGN-EXAMINATION
IN VITRO DIAGNOSTIC MEDICAL DEVICES

GB HIV Ag-Ab COMB for qualitative screening in human serum or plasma (EDTA, Heparin and Citrate) of antibodies to HIV-1, HIV-2 and HIV-1 p24 antigen

Issued to:

General Biologicals Corporation
No. 6 Innovation First Road Hsinchu Science Park Baoshan Township, Taiwan, R.O.C.
30076 Hsinchu County 30076
Taiwan

This certificate covers the following product(s):

Product variants:
Product code: 4EAIC11 (96 tests)
Product code: 4EAIC13 (480 tests)

Initial date: 12 February 2020

DEKRA Certification B.V.

B.T.M. Holtus
Managing Director

J.A. van Vugt
Certification Manager

DEKRA Certification B.V. is Notified Body with ID no 0344

DEKRA Certification B.V. Meander 1051, 6825 MJ Arnhem P.O. Box 5185, 6802 ED Arnhem, The Netherlands
T +31 88 96 83000 F +31 88 96 83100 www.dekra.nl Company registration 09085396

Anlage 2
(zu § 4 Abs. 1 Nr. 1 DIMDIV)
Formularnummer 00152828

Allgemeine Anzeigepflicht nach §§ 25 und 30 Abs. 2 MPG
General Obligation to Notify pursuant to §§ 25 and 30 (2) Medical Devices Act, MPG

Formblatt für In-vitro-Diagnostika / Form for In Vitro Diagnostic Medical Devices

Zuständige Behörde / Competent authority			
	Code **DE/CA70**		
	Bezeichnung / Name **Landesamt für Umwelt- und Arbeitsschutz**		
	Staat / State **Deutschland**		Land / Federal state **Saarland**
	Ort / City **Saarbrücken**		Postleitzahl / Postal code **66119**
	Straße, Haus-Nr. / Street, house no. **Don-Bosco-Straße 1**		
	Telefon / Phone **+49-681-85000**		Telefax / Fax **+49-681-85001384**
	E-Mail / E-mail **lua@lua.saarland.de**		

Anzeige / Notification			
	Registrierdatum bei der zuständigen Behörde Registration date at competent authority **27.01.2020**		Registriernummer / Registration number **DE/CA70/40838-152828**
	Typ der Anzeige / Notification type £ Erstanzeige / Initial notification S Änderungsanzeige / Notification of change £ Widerrufsanzeige / Notification of withdrawal		
	Frühere Registriernummer bei Änderungs- und Widerrufsanzeige Previous registration number if notification has been changed or withdrawn **DE/CA70/40838-56402**		
	Anzeigender nach § 25 MPG / Reporter pursuant to § 25 Medical Devices Act, MPG £ Hersteller / Manufacturer S Bevollmächtigter / Authorised Representative £ Einführer / Importer £ Verantwortlicher für das Zusammensetzen von Systemen oder Behandlungseinheiten nach § 10 Abs. 1 und 2 MPG \ Assembler of systems or procedure packs pursuant to § 10 (1) and (2) Medical Devices Act, MPG £ Betrieb oder Einrichtung (aufbereiten) nach § 25 Abs. 1 MPG i. V. m. § 4 Abs. 2 MPBetreibV Institution (processing) pursuant to § 25 (1) Medical Devices Act, MPG in connection with § 4 (2) MPBetreibV £ Betrieb oder Einrichtung (sterilisieren) nach § 25 Abs. 2 i. V. m. § 10 Abs. 3 MPG Institution (sterilizing) pursuant to § 25 (2) in connection with § 10 (3) Medical Devices Act, MPG		

Anzeigender / Reporting organisation (person)			
	Code **DE/0000040838**		
	Bezeichnung / Name **Medical Technology Promedt Consulting GmbH**		
	Staat / State **Deutschland**		Land / Federal state **Saarland**
	Ort / City **ST. INGBERT**		Postleitzahl / Postal code **66386**
	Straße, Haus-Nr. / Street, house no. **Altenhofstrasse 80**		
	Telefon / Phone **+49-6894-581020**		Telefax / Fax **+49-6894-581021**
	E-Mail / E-mail **info@mt-procons.com**		

Hersteller / Manufacturer			
	Bezeichnung / Name **General Biologicals Corporation**		
	Staat / State **TW**		
	Ort / City **Hsinchu County**		Postleitzahl / Postal code **30076**
	Straße, Haus-Nr. / Street, house no. **No.6, Innovation First Road, Hsinchu Science Park, Baoshan Township**		
	Telefon / Phone **+886 3 5779221**		Telefax / Fax
	E-Mail / E-mail		

Sicherheitsbeauftragter für Medizinprodukte nach § 30 Abs. 2 MPG 9) **Safety officer for medical devices pursuant to § 30 (2) Medical Devices Act, MPG**			
	Bezeichnung / Name **Dr. Michael Rinck**		
	Staat / State **Deutschland**		Land / Federal state **Saarland**
	Ort / City **ST. INGBERT**		Postleitzahl / Postal code **66386**
	Straße, Haus-Nr. / Street, house no. **Altenhofstrasse 80**		
	Telefon / Phone **+49-6894-581020**		Telefax / Fax **+49-6894-581021**
	E-Mail / E-mail **info@mt-procons.com**		

Vertreter / Deputy (optional)			
	Bezeichnung / Name **Clemens Mohr**		
	Telefon / Phone **+49-6894-581020**		Telefax / Fax **+49-6894-581021**
	E-Mail / E-mail **info@mt-procons.com**		
	£ Erstanzeige / Initial notification S Änderungsanzeige / Notification of change		

In-vitro-Diagnostikum / In vitro diagnostic medical device	
	Klassifizierung / Classification £ Produkt der Liste A, Anhang II / Device of List A, Annex II £ Produkt der Liste B, Anhang II / Device of List B, Annex II £ Produkt zur Eigenanwendung / Device for self-testing S Sonstiges Produkt / Other device (all devices except Annex II and self-testing devices)
	App (Software auf mobilen Endgeräten) £ ja / yes S nein / no
	Anzeige nach § 25 Abs. 3 Nummer 3 MPG Notification pursuant to § 25 (3) number 3 Medical Devices Act, MPG £ "Neues In-vitro-Diagnostikum / New in vitro diagnostic medical device"
	Handelsname des Produktes / Trade name of the device **HEPAVASE MA-96(TMB)**
	Produktbezeichnung / Name of device
	Angabe der benutzten Nomenklatur / Nomenclature used S EDMS-Klassifikation / EDMS Classification £ GMDN
	Nomenklaturcode / Nomenclature code **15-02-01-06-00**
	Nomenklaturbezeichnung / Nomenclature term **HAV ANTIBODY IGM**
	Kurzbeschreibung / Short description In Deutsch / In German **HEPAVASE MA-96 (TMB) ist ein enzymatisches Immunoassay Diagnostik-Kit für die qualitative in-vitro Detektion von Anti-HAV IgM in humanem Serum oder Plasma. Interne Meldenr.: GBC-05.**
	In Englisch / In English **HEPAVASE MA-96 (TMB) is an enzyme immunoassay diagnostic kit, for in vitro qualitative detection of Anti-HAV IgM in human serum or plasma. Internal Admin No.:GBC-05.**

Zusätzliche Angaben im Falle der In-vitro-Diagnostika gemäß Anhang II und der In-vitro-Diagnostika zur Eigenanwendung / Addtional information for Annex II and self-testing in vitro diagnostic medical devices	
	Nummer(n) der Bescheinigung(en) / Certificate number(s)
	£ In Übereinstimmung mit den Gemeinsamen Technischen Spezifikationen (für Produkte gem. Anhang II, Liste A) In conformity with Common Technical Specifications (for Annex II List A devices)
	Ergebnisse der Leistungsbewertung Outcome of performance evaluation

Ich versichere, dass die Angaben nach bestem Wissen und Gewissen gemacht wurden.
I affirm that the information given above is correct to the best of my knowledge.

Ort
City **St. Ingbert**

Datum
Date **2020-01-22**

Name **Sabrina Neumann**

Unterschrift
Signature

Bearbeitungsvermerke / Processing notes Nur von der zuständigen Behörde auszufüllen / To be filled in only by the competent authority			
	Bearbeiter / Person responsible **Herr Josef Opitz**		Telefon / Phone **0681 8500-1330**

Anlage 2
(zu § 4 Abs. 1 Nr. 1 DIMDIV)
Formularnummer 00152642

Allgemeine Anzeigepflicht nach §§ 25 und 30 Abs. 2 MPG
General Obligation to Notify pursuant to §§ 25 and 30 (2) Medical Devices Act, MPG

Formblatt für In-vitro-Diagnostika / Form for In Vitro Diagnostic Medical Devices

Zuständige Behörde / Competent authority			
	Code **DE/CA70**		
	Bezeichnung / Name **Landesamt für Umwelt- und Arbeitsschutz**		
	Staat / State **Deutschland**		Land / Federal state **Saarland**
	Ort / City **Saarbrücken**		Postleitzahl / Postal code **66119**
	Straße, Haus-Nr. / Street, house no. **Don-Bosco-Straße 1**		
	Telefon / Phone **+49-681-85000**		Telefax / Fax **+49-681-85001384**
	E-Mail / E-mail **lua@lua.saarland.de**		

Anzeige / Notification			
	Registrierdatum bei der zuständigen Behörde Registration date at competent authority **17.01.2020**		Registriernummer / Registration number **DE/CA70/40838-152642**
	Typ der Anzeige / Notification type £ Erstanzeige / Initial notification S Änderungsanzeige / Notification of change £ Widerrufsanzeige / Notification of withdrawal		
	Frühere Registriernummer bei Änderungs- und Widerrufsanzeige Previous registration number if notification has been changed or withdrawn **DE/CA70/40838-56383**		
	Anzeigender nach § 25 MPG / Reporter pursuant to § 25 Medical Devices Act, MPG £ Hersteller / Manufacturer S Bevollmächtigter / Authorised Representative £ Einführer / Importer £ Verantwortlicher für das Zusammensetzen von Systemen oder Behandlungseinheiten nach § 10 Abs. 1 und 2 MPG \ Assembler of systems or procedure packs pursuant to § 10 (1) and (2) Medical Devices Act, MPG £ Betrieb oder Einrichtung (aufbereiten) nach § 25 Abs. 1 MPG i. V. m. § 4 Abs. 2 MPBetreibV Institution (processing) pursuant to § 25 (1) Medical Devices Act, MPG in connection with § 4 (2) MPBetreibV £ Betrieb oder Einrichtung (sterilisieren) nach § 25 Abs. 2 i. V. m. § 10 Abs. 3 MPG Institution (sterilizing) pursuant to § 25 (2) in connection with § 10 (3) Medical Devices Act, MPG		

Anzeigender / Reporting organisation (person)			
	Code **DE/0000040838**		
	Bezeichnung / Name **Medical Technology Promedt Consulting GmbH**		
	Staat / State **Deutschland**		Land / Federal state **Saarland**
	Ort / City **ST. INGBERT**		Postleitzahl / Postal code **66386**
	Straße, Haus-Nr. / Street, house no. **Altenhofstrasse 80**		
	Telefon / Phone **+49-6894-581020**		Telefax / Fax **+49-6894-581021**
	E-Mail / E-mail **info@mt-procons.com**		

Hersteller / Manufacturer			
	Bezeichnung / Name **General Biologicals Corporation**		
	Staat / State **TW**		
	Ort / City **Hsinchu County**		Postleitzahl / Postal code **30076**
	Straße, Haus-Nr. / Street, house no. **No.6, Innovation First Road, Hsinchu Science Park, Baoshan Township**		
	Telefon / Phone **+886 3 5779221**		Telefax / Fax
	E-Mail / E-mail		

Sicherheitsbeauftragter für Medizinprodukte nach § 30 Abs. 2 MPG 9) **Safety officer for medical devices pursuant to § 30 (2) Medical Devices Act, MPG**			
	Bezeichnung / Name **Dr. Michael Rinck**		
	Staat / State **Deutschland**		Land / Federal state **Saarland**
	Ort / City **ST. INGBERT**		Postleitzahl / Postal code **66386**
	Straße, Haus-Nr. / Street, house no. **Altenhofstrasse 80**		
	Telefon / Phone **+49-6894-581020**		Telefax / Fax **+49-6894-581021**
	E-Mail / E-mail **info@mt-procons.com**		

Vertreter / Deputy (optional)

Bezeichnung / Name
Clemens Mohr

Telefon / Phone
+49-6894-581020

Telefax / Fax
+49-6894-581021

E-Mail / E-mail
info@mt-procons.com

£ Erstanzeige / Initial notification
S Änderungsanzeige / Notification of change

In-vitro-Diagnostikum / In vitro diagnostic medical device

Klassifizierung / Classification
£ Produkt der Liste A, Anhang II / Device of List A, Annex II
£ Produkt der Liste B, Anhang II / Device of List B, Annex II
£ Produkt zur Eigenanwendung / Device for self-testing
S Sonstiges Produkt / Other device (all devices except Annex II and self-testing devices)

App (Software auf mobilen Endgeräten) £ ja / yes S nein / no

Anzeige nach § 25 Abs. 3 Nummer 3 MPG
Notification pursuant to § 25 (3) number 3 Medical Devices Act, MPG
£ "Neues In-vitro-Diagnostikum / New in vitro diagnostic medical device"

Handelsname des Produktes / Trade name of the device
HEPAVASE A-96 (TMB)

Produktbezeichnung / Name of device

Angabe der benutzten Nomenklatur / Nomenclature used
S EDMS-Klassifikation / EDMS Classification
£ GMDN

Nomenklaturcode / Nomenclature code
15-02-01-04-00

Nomenklaturbezeichnung / Nomenclature term
HAV ANTIBODY (TOTAL)

Kurzbeschreibung / Short description
In Deutsch / In German
HEPAVASE A-96 (TMB) ist ein enzymatisches Immundiagnostik-Kit für die qualitative in vitro Detektion von Anti-HAV in humanem Serum oder Plasma. Interne Meldenr.:GBC-03

In Englisch / In English
HEPAVASE A-96 (TMB) is an enzyme immunoassay diagnostic kit, for in vitro qualitative detection of Anti-HAV in human serum or plasma. Internal admin No.: GBC-03

Zusätzliche Angaben im Falle der In-vitro-Diagnostika gemäß Anhang II und der In-vitro-Diagnostika zur Eigenanwendung / Addtional information for Annex II and self-testing in vitro diagnostic medical devices	
	Nummer(n) der Bescheinigung(en) / Certificate number(s)
	£ In Übereinstimmung mit den Gemeinsamen Technischen Spezifikationen (für Produkte gem. Anhang II, Liste A) In conformity with Common Technical Specifications (for Annex II List A devices)
	Ergebnisse der Leistungsbewertung Outcome of performance evaluation

Ich versichere, dass die Angaben nach bestem Wissen und Gewissen gemacht wurden.
I affirm that the information given above is correct to the best of my knowledge.

Ort
City **St. Ingbert**

Datum
Date **2020-01-10**

Name **Sabrina Neumann**

Unterschrift
Signature

Bearbeitungsvermerke / Processing notes Nur von der zuständigen Behörde auszufüllen / To be filled in only by the competent authority			
	Bearbeiter / Person responsible **Frau Caroline Bauer**		Telefon / Phone **0681 8500-1198**

Certificate of Registration

QUALITY MANAGEMENT SYSTEM - ISO 13485:2016 & EN ISO 13485:2016

This is to certify that:

General Biologicals Corporation
No. 6, Innovation First Road
Hsinchu Science Park
Baoshan Township
Hsinchu County
300
Taiwan

Holds Certificate Number: MD 93006

and operates a Quality Management System which complies with the requirements of ISO 13485:2016 & EN ISO 13485:2016 for the following scope:

The design, manufacture and sales of in vitro diagnostics tests for the detection of hepatitis virus, HIV, tumor and biomarkers using immunoassay and molecular diagnostic reagents. The sales of in vitro diagnostics test kits for the detection of anemia, fertility hormone, growth factor (HGH), infectious disease, kidney function, tumor markers, thyroid hormones, cardiac markers, fecal occult blood, blood glucose and related instruments using immunoassay, chemiluminescence immunoassay, rapid test and nucleic acid testing.

For and on behalf of BSI:

Graeme Tunbridge, Senior Vice President Global Regulatory & Quality

Original Registration Date: 2005-04-26
Latest Revision Date: 2024-10-31

Effective Date: 2025-01-07
Expiry Date: 2028-01-06

...making excellence a habit.™

GBC General Biologicals Corporation

No. 6, Innovation First Road, Hsinchu Science Park,
Hsinchu 30076, Taiwan, R.O.C.
Tel +886.3.5779.221 Fax +886.3.5779.227

Product Name	HEPAVASE A-96 (TMB)		
IVD	Product Code	4AGE3 4AGE 11	CE
Classification	Not listed in **Annex II** List A or List B of 98/79/EC (IVDD)		
Intended Use	For qualitative detection of antibodies to hepatitis A virus (Anti-HAV) in human serum or plasma		

Legal manufacturer	GENERAL BIOLOGICALS CORPORATION
Address	No. 6, INNOVATION FIRST ROAD, HSINCHU SCIENCE PARK, BAOSHAN TOWNSHIP, HSINCHU COUNTY 30076, TAIWAN, R.O.C..
Tel.	+886-3-5779221
Fax.	+886-3-5779227
E mail	sales.group@gbc.com.tw

Authorized Representative in EC	Medical Technology Promedt Consulting GmbH
Address	Altenhofstrasse 80, 66386 St. Ingbert, Germany
Tel.	+49 6894-581020
Fax.	+49 6894-581021
E mail	info@mt-procons.com

Manufacturing Facility incl. Places of final testing	**Research and Development Facility**
GENERAL BIOLOGICALS CORP. #6, INNOVATION FIRST ROAD, SCIENCE PARK, HSINCHU, TAIWAN, R.O.C.	GENERAL BIOLOGICALS CORP. #6, INNOVATION FIRST ROAD, SCIENCE PARK, HSINCHU, TAIWAN, R.O.C.

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1. Intended Use

HEPAVASE A-96 (TMB) is an enzyme immunoassay for in vitro qualitative detection of antibody to hepatitis A virus (Anti-HAV) in human serum or plasma (heparin, EDTA or citrate).

2. Summary and Test Explanation

The hepatitis A virus (HAV) is a single-stranded RNA-containing virus without an envelope and with a diameter of 27 nm that belongs to the family of Picornaviridae [*1]. Hepatitis A - the most common form of acute viral hepatitis - is an infection of fecal-oral transmission produced in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice [*2].

Total anti-HAV and especially IgM anti-HAV is positive at the onset of a hepatitis A infection. After natural infection, anti-HAV-IgG antibodies can usually be detected for a lifetime providing protection against the disease [*3-4]. The detection of anti-HAV is indicative of current immunity and helps in deciding whether active immunization should be supplied by vaccination or immunoglobulins should be administered for post-exposure prophylaxis in at-risk situations [*5-6].

HEPAVASE A-96 (TMB) is a fast test for the qualitative detection of antibodies to Hepatitis A virus in serum or plasma (heparin, citrate or EDTA) specimens. This is an enzyme linked immunosorbent assay (ELISA) which utilizes HAV Ag on microtiter wells and human peroxidase-conjugated Anti-HAV in a competition principle to detect Anti-HAV levels in serum or plasma.

Specimens with absorbance values greater than the Cutoff Value are considered **NONREACTIVE** for Anti-HAV

Specimens with absorbance values lower or equal than the Cutoff Value are considered **REACTIVE** for Anti-HAV.

The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above.

If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples of the patient.

3. Test Description

HEPAVASE A-96 (TMB) is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immunosorbent assay) based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HAV Ag and the liquid phase of human peroxidase conjugated Anti-HAV.

When a serum or plasma specimen containing Anti-HAV is added to the HAV Ag-coated wells together with the human peroxidase conjugated Anti-HAV and incubated, a competition will take place for the binding to the HAV Ag on the wells. (HAV Ag)-(Anti-HAV • Peroxidase) complex and/or (HAV Ag)-(Anti-HAV) complex will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HAV bound to HAV Ag deriving from the specimen. The Peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm [*7] .

A Specimen containing Anti-HAV:

1. Plate well (HAV Ag) + specimen (Anti-HAV) + Anti-HAV·Peroxidase

	→ Plate-HAV Ag-Anti-HAV complex and/or Plate-HAV Ag-Anti-HAV·Peroxidase complex 2. Washing to remove unbound material 3. Add TMB substrate solution → blue color to light pale blue color/even colorless 4. Add sulfuric acid to stop the color development → Read OD at 450nm with a selected reference wavelength within 620 to 690nm[*7] B Specimen without Anti-HAV: 1. Plate well (HAV Ag) + specimen (without Anti-HAV) + Anti-HAV·Peroxidase → Plate-HAV Ag-Anti-HAV·Peroxidase complex 2. Wash to remove the unbound material. 3. Add TMB substrate solution → colorless to light blue color 4. Add 2N sulfuric acid to stop the color development, read OD at 450nm with a selected reference wavelength within 620 to 690nm[*7]

4. Description of Materials Provided & Product Code System

● **Storage Conditions:** Item 1 - 6 on the following reagent table should be refrigerated at +2 to 8+ °C and the others can be stored at +2 to +30 °C.

ITEMS	Material Code	Components	Description	Qt. per 96 tests	Qt. per 480 tests
(1)	3A040MP	HAV Antigens Plate	Microtiter Plate Coated with HAV Antigen.	1 plate	5 plates
(2)	3A071-G3	Anti-HAV·Peroxidase Solution	Anti-HAV (mouse monoclonal) ·Peroxidase (horseradish) conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1bottle, 12 ml	5bottles, 12 ml
(3)	3A090-G3	CONTROL + Anti-HAV Positive Control	Human plasma positive for antibody to HAV in buffer with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle, 1 ml	2bottles, 1 ml
(4)	3A110-G3	CONTROL - HAV Negative Control	Human plasma non-reactive for antibody to HAV with protein stabilizers. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.	1 bottle, 1 ml	2bottles, 1 ml
(5)	3B135TMB-A	TMB Substrate Solution A	0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle, 12 ml	3bottles, 12 ml
(6)	3B140TMB-B	TMB Substrate Solution B	Citric Acid Buffer containing 0.03% H_2O_2.	1 bottle, 12 ml	3bottles, 12 ml
(7)	3B112PBS3	Conc. Washing Solution D (20X)	Concentrated phosphate buffer with Tween-20.	1 bottle 58 ml	5bottles, 58 ml
(8)	3B155SACID2N	Stop Solution 2	2N H_2SO_4 (Sulfuric Acid)	1 bottle 12 ml	5bottles, 12 ml

● ACCESSORIES: (provided as needed)

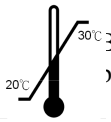
ITEMS	Material Code	Components
(9)	2P951001	Adhesive Slips
(10)	2P505001	Absorbent Pads
(11)	2P403001	Black Cover

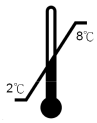
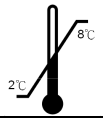
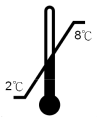
● OTHER MATERIALS AND DEVICES REQUIRED, BUT NOT PROVIDED

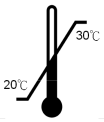
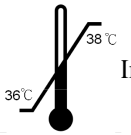
ITEMS	Components
(1)	10µl, 100µl and 1.0 ml micropipettes and tips are needed
(2)	Incubator or waterbath with temperature control at +37 °C.
(3)	Plate washing equipment.
(4)	ELISA microwell reader: Dual wavelength 450nm with 620-690nm as reference wavelength[*7], bandwidth 10nm
(5)	Purified water: distilled or deionized water.
(6)	Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

4.1. Storage Conditions and Stability of Kit and Components *

Kit/Components	Storage condition	State	Stability
HEPAVASE A-96 (TMB) KIT	+2～+8 °C	Original	15 months
		Once open	1 month
Anti-HAV Positive Control	+2～+8 °C	Original	15 months
		Once open	1 month
HAV Negative Control	+2～+8 °C	Original	15 months
		Once open	1 month
HAV Antigens Plate	+2～+8 °C	Original	15 months
		Once open	2 months
Anti-HAV · Peroxidase Solution	+2～+8 °C	Original	15 months
		Once open	1 month
Conc. Washing Solution D (20x)	Room temp.	Original	24 months
		Once open	1 month
20X Diluted Washing Solution	Room temp.	Diluted	2 days
	+2～+8 °C	Diluted	1 week
TMB Substrate Solution A	+2～+8 °C	Original	24 months
		Once open	1 month
TMB Substrate Solution B	+2～+8 °C	Original	24 months
		Once open	1 month
Stop Solution 2	Room temp.	Original	24 months
		Once open	1 month

5.	**Instructions for Use**
5.1.	**Warnings**
5.1.1.	This reagent kit is for professional use only.
5.1.2.	IVD This reagent kit is for *in vitro* diagnostic use only.
5.1.3.	 Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix gently before use.
5.1.4.	 Do not use reagent beyond its expiration date.
5.1.5.	Do not interchange reagents between different lots.
5.1.6.	Do not pipette in the mouth.
5.1.7.	Do not smoke or eat in areas where specimens or reagents are handled.
5.1.8.	The positive control, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They shall be used and discarded according to the user's laboratory safety procedures. Such safety procedures probably shall include wearing protective gloves and avoiding aerosols generation.
5.1.9.	Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.
5.1.10.	**Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows:** Both liquid and solid waste should be autoclaved maintaining +121 °C for at least 30 minutes. Solid waste can also be incinerated. Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%. Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
5.1.11. 	2N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.
5.1.12. 	TMB substrate solution A contains an organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.

5.2.	**Specimen Collection and Preparation for Analysis**
5.2.1.	No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.
5.2.2.	Either serum or plasma can be used with this diagnostic kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
5.2.3.	 Specimens must be stored at +2 to +8 °C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20 °C. Storage in self-defrosting freezer is not recommended.
5.2.4.	Frozen specimens must be thoroughly thawed and mixed homogenously before test.
5.2.5.	Avoid multiple freeze-thaw procedures
5.2.6.	
⚠ **WARNING**	1. The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity. 2. Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.
5.3.	**Reagents storage**
5.3.1.	 The kit must be stored at +2 to +8°C. Do not freeze.
5.3.2.	Strips of the plate should be used within 1 month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.
5.3.3.	 Return the reagents to +2 to +8 °C immediately after use.
5.3.4	Conc. Washing Solution D (20X) should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a +37 °C water bath till the crystal is dissolved.
5.4.	**Plate washing procedure**
5.4.1.	Preparation of washing solution: Dilute Conc. Washing Solution D (20X) with distilled or de-ionized water to 1:20 dilution. Do not use tap water.
5.4.2.	Plate washing: Any commercial automatic micro-plate washer or other liquid aspirating/ dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing. **It is suggested to wash 6 cycles with at least 350µl washing buffer per well per wash and soaking at least for 10 seconds.**
5.4.3.	Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.
⚠ **WARNING**	Improper washing will cause false results.

5.5.	**Test procedure**
	Assay process can be performed by an automatic EIA micro-plate immunoanalyzer,. Please set up the program according to the following test procedure.
5.5.1.	 Bring all reagents and specimens to room temperature (+20 to +30°C) before assay. Adjust water bath or incubator to +37±1°C.
5.5.2.	Prepare the needed number of wells, including 2 wells for blanks, 3 wells for Negative Control, 2 wells for Positive Control, and one well for each specimen. Reserve 2 wells for blanks (**Do not add any specimen or conjugate)**. Add 10µl of each control or specimen to the appropriate wells of HAV Ag coated plate, except the 2 blanks. **NOTE:** a. Use a new pipette tip for each sampling to avoid cross-contamination b. Each plate needs its own negative controls, positive controls and blank wells. c. Do not use cut-off values established for other plates of HEPAVASE A-96 (TMB).
5.5.3.	Add 100µl of Anti-HAV·Peroxidase solution to each of the above wells except the 2 blanks. **Note:** Do not touch the cuvette wall for preventing contamination.
5.5.4.	Gently tap the plate.
5.5.5.	Seal the plate with an adhesive slip.
5.5.6.	 Incubate the reaction plate in +**37±1 °C** water bath or incubator for **one hour**.
5.5.7.	At the end of the incubation period, remove and discard the adhesive slip and wash the plate in accordance with **5.4) Plate washing procedure.**
5.5.8.	Choice one of the following two methods for color development: **NOTE:** TMB Substrate Solution A should be colorless to light blue, otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mixing. The mixture should be avoided from intense light. A. Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 µl of the mixture solution to each well including the 2 blank wells. B. Add 50µl of TMB Substrate Solution A first, then add 50µl of TMB Substrate Solution B into each well including the 2 blanks. Mix well gently.
5.5.9.	Cover the plate with black cover and incubate at room temperature for 30 minutes.
5.5.10.	Stop the reaction by adding 100 µl of Stop Solution 2 to each well including the blank.
5.5.11.	Determine the absorbance of controls and test specimens within 15 minutes with a photometer at 450nm with a selected reference wavelength within 620 to 690nm[*7]. Use the blank well to blank the photomer. **NOTE:** The color of the blank should be colorless to light yellowish; otherwise, the test result is invalid. In this case the test must be repeated. Substrate blank: absorbance value must be less than 0.100.

5.6.	**Calculation of Test Results**
5.6.1.	Calculation of the NCx (Mean Absorbance of Negative Control). Example: Sample No. Absorbance 1 1.263 2 1.305 3 1.290 NCx = (1.263 + 1.305 + 1.290)/3=1.286 **NCx must be ≥ 0.4, otherwise, the test is invalid.**
5.6.2.	Calculation of PCx (Mean Absorbance of Positive Control) Example: Sample No. Absorbance 1 0.054 2 0.060 PCx = (0.054 + 0.060) /2 = 0.057 **PCx must be ≤0.1, otherwise, the test is invalid.**
5.6.3.	Calculation of the **N-P Value** **N-P = NCx – PCx** Example: N - P = 1.286 - 0.057 = 1.229 **N-P Value must be ≥ 0.3, otherwise, the test is invalid.**
5.6.4.	Calculation of the **Cutoff Value** **Cutoff Value = (NCx + PCx)/2** Example: Cutoff Value =(1.286 + 0.057)/2 = 0.672
5.6.5.	Calculation of the **Retest Range** **Retest Range = Cutoff Value ±10%** Example: Cutoff Value = 0.672 Retest Range = (0.672 - 0.067) to (0.672 + 0.067) = 0.605 to 0.739
5.7.	**Validity of Test Runs**
5.7.1.	**NCx must be ≥0.4, otherwise, the test is invalid.**
5.7.2.	**PCx must be ≤0.1, otherwise, the test is invalid.**
5.7.3.	**N-P Value must be ≥0.3, otherwise, the test is invalid.**

5.8.	**Interpretation of Results**
5.8.1.	Specimens with O.D. values **GREATER** than the **Cutoff Value** are considered **non-reactive** for Anti-HAV.
5.8.2.	Specimens with O.D. values **LOWER** than or **EQUAL** to the **Cutoff Value** are considered **reactive** for Anti-HAV.
5.8.3.	If the data is within the **Retest Range**, the test must be repeated in duplicate and interpreted as above. If the retested absorbance still within the retest range, it is suggested to test follow-up-samples.
5.9.	**Troubleshooting**
	If the result cannot be reproduced, a preliminary troubleshooting should be performed by checking the possibilities listed below:
5.9.1.	Improper washing procedure.
5.9.2.	Contamination with positive specimens.
5.9.3.	Wrong volume of sample, conjugate or substrates.
5.9.4.	Contamination of well rim with conjugate.
5.9.5.	Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not being mixed well before use.
5.9.6.	Wrong incubation time or temperature.
5.9.7.	Obstructed or partial obstructed washer aspirate/dispense head and needles.
5.9.8.	Insufficient aspiration.
5.10.	**Limitations and Interferences**
5.10.1.	**This reagent kit is to be used for un-pooled human serum or plasma samples only.**
5.10.2.	**Non-repeatable reactive results may be obtained with any** enzyme immunoassay **kit,** largely due to technical error e**i**ther on the part of the operator or malfunction of apparatus used.
5.10.3.	The reagent kit has not been validated for use with cadaveric samples.
5.10.4.	Potential interfering substances: By addition tests the following results were obtained: 1. The anticoagulants heparin, citrate and EDTA had no effect on the test result. 2. Hemoglobin up to 8.0 g/l had no effect on the test result. 3. Bilirubin up to 0.3 g/l: had no effect on the test result. 4. Triglyceride up to 5.0 g/l had no effect on the test result. 5. A rheumatoid factor high positive specimen exhibited a false positive result. 6. Pregnancy did not affect the test result.

5.11. Performance Characteristics

5.11.1. Diagnostic Sensitivity and Diagnostic Specificity

1. Specimens from hospitalized patients:

		GBC HEPAVASE A-96 (TMB)		
		Negative	Positive	Total
Comparison assay	Negative	984	5	989
	Positive	1	551	552
	total	985	556	1541

Diagnostic sensitivity = 100% x 551/552 = 99.8%

Diagnostic specificity = 100% x 984/989 = 99.5%

2. Patients with acute hepatitis A:

		GBC HEPAVASE A-96 (TMB)		
		Negative	Positive	Total
Comparison assay	Negative	42	0	42
	Positive	0	9	9
	total	42	9	51

Conformity = 100%

3. Hepatitis A patients in convalescent period:

		GBC HEPAVASE A-96 (TMB)		
		Negative	Positive	Total
Comparison assay	Negative	10	0	10
	Positive	0	18	18
	total	10	18	28

Conformity = 100%

4. Hepatitis B carriers:

		GBC HEPAVASE A-96 (TMB)		
		Negative	Positive	Total
Comparison assay	Negative	85	0	85
	Positive	0	22	22
	total	85	22	107

Conformity = 100%

5. Auto-immune patients:

		GBC's HEPAVASE A-96 (TMB)		
		Negative	Positive	Total
Comparison assay	Negative	4	0	4
	Positive	0	16	16
	total	4	16	20

Conformity = 100%

6. Patients with HAV infection:

		GBC HEPAVASE A-96 (TMB)		
		Negative	Positive	Total
Comparison assay	Negative	0	0	0
	Positive	0	19	19
	total	0	19	19

Diagnostic specificity = 100%

Diagnostic sensitivity = 100%

7. Patients with other viral infections:

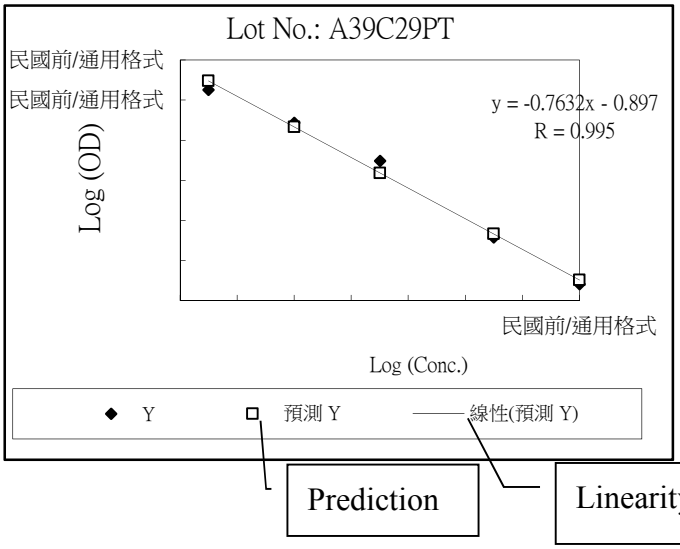
		GBC HEPAVASE A-96 (TMB)		
		Negative	Positive	Total
Comparison assay	Negative	15	0	15
	Positive	0	20	20
	total	0	0	35

Conformity = 100%

5.11.2. Analytical Sensitivity

Analytical sensitivity = 0.121 PEI U/ml 0.157 IU/ml.

Conc. (PEI U/ml)	OD	Log (Conc.)	Log (OD)
1	0.121	0	-0.9172146
0.5	0.207	-0.30103	-0.6840297
0.2	0.499	-0.69897	-0.3018995
0.1	0.77	-1	-0.1135093
0.05	1.125	-1.30103	0.0511525
Cutoff	0.636	-0.91776471	-0.1965429
Sensitivity	0.121 PEI U/ml		



5.11.3.	**Precision**
	Intra-assay reproducibility: Intra-assay CV% < 20
	Inter-assay reproducibility: Inter-assay CV% <25
5.11.4.	**Traceability:**
	Concentration of Anti-HAV Positive Control = 7 ±4 PEI U/ml = 9.1 ±5.2 IU/ml

5.12.	**Flow chart of the test procedure**
	The simplified procedure should be used only by experienced users. New users are advised to read and follow the detailed test procedure carefully.

Add 10µl controls (3xNC, 2xPC) and add 10µl of each specimen into wells. Reserve 2 wells for the blanks.

↓

Add 100µl of Anti-HAV·Peroxidase solution into each reaction well, except 2 blanks.

↓

Incubate the plate at +37±1°C for 1 hour

↓

Wash the plate

(Choose one of the following two methods for color development)

/ \

Mix equal volumes of TMB Substrate Solution A and B. Add 100µl of the mixed solution to wells.

Add 50µl of TMB Substrate Solution A to wells and then add 50µl of TMB Substrate Solution B. Mix well, gently.

\ /

Incubate at R.T. for 30 minutes.

↓

Add 100µl of Stop Solution 2 into each well.

↓

Determine absorbance using 450 nm as reading wavelength with 620-690 nm reference wavelength[*7].

6. Bibliography

1. Melnick JL. History and epidemiology of hepatitis A virus. J Infect Dis 1995;171(Suppl 1):2-8.
2. Koff RS. Hepatitis A. Lancet 1998;341:1643–49.
3. Lemon SM, Binn LN. Serum neutralizing antibody response to hepatitis A virus. J Infect Dis. 1983;148**:** 1033-1039.
4. Duermeyer W., Van der Veen J., Koster B. "ELISA in Hepatitis A". Lancet. 1978; 1(8068).:823-824.
5. Lemon SM. Inactivated hepatitis A virus vaccines. Hepatology.1992;15:1194-1197.
6. Craig AS, Schaffner W. Prevention of hepatitis A with the hepatitis A vaccine N Engl J Med 2004; 350:476-481
7. The reference wavelength of spectrometer can be 620nm to 690nm. However, user should validate the photometer in combination with this kit before use.

END OF THE INSTRUCTIONS FOR USE.

	Symbols Key / Symbolschlussel / Explication des Symboles / Interpretazione simboli / Clave dos Simbolos
	Manufacturer / Hersteller / Fabricante / Fabbricante / Fabricant / Fabricante
IVD	In Vitro Diagnostic Medical Device / In-Vitro-Diagnostikum / Producto sanitario para diagnóstico in vitro / Dispositivo medico-diagnostico in vitro / Dispositif médical de diagnostic in vitro / Dispositivo médico para diagnóstico in vitro
LOT	Batch code / Chargenbezeichnung / Código de lote / Codice del lotto / Code du lot / Código do lote
	Use By / Verwendbar bis / Fecha de caducidad / Utilizzare entro / Utiliser jusque / Prazo de validade
	Temperature limitation / Temperaturbegrenzung / Límite de temperatura / Limiti di temperatura / Limites de température / Limites de temperatura
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / CE Marca / Marca CE
REF	Catalogue number / Bestellnummer / Número de catálogo / Numero di catalogo / Référence du catalogue / Referência de catálogo
	Consult Instructions for Use- / Gebrauchsanweisung beachten / Consulte las instrucciones de uso / Consultare le istruzioni per l'uso / Consulter les instructions d'utilisation / Consulte as instruções de utilização
	Caution,consult accompanying documents / Achtung, Begleitdokumente beachten / Atención,ver instrucciones de uso / Attenzione, vedere le istruzioni per l'uso / Attention voir notice d'instructions / Atenção, consulte a documentação incluída
EC REP	Authorized representative/ Bevollmzed repre Vertreter / repreprer / autoriss/ Rappresentante autorizzato/ Reprezentant autorizat/ Representante autorizado

GBC General Biologicals Corporation

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Tel +886.3.5779.221 Fax +886.3.5779.227

Product Name	HEPAVASE MA-96 (TMB)		
IVD	Product Code	4AME3 4AME11	CE
Classification	Not listed in Annex II List A or List B of 98/79/EC (IVDD)		
Intended Use	For in-vitro qualitative detection of IgM antibody to hepatitis A virus (Anti-HAV IgM) in human serum or plasma		

Legal manufacturer	**GENERAL BIOLOGICALS CORPORATION**
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Authorized Representative in EC	**Medical Technology Promedt Consulting GmbH**
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Tel.	+49 (0) 6894-581020
Fax.	+49 (0) 6894-581021
E mail	info@mt-procons.com

Manufacturing Facility incl. Places of final testing	**Research and Development Facility**
No. 6, INNOVATION FIRST ROAD, HSINCHU SCIENCE PARK, BAOSHAN TOWNSHIP, HSINCHU COUNTY 30076, TAIWAN, R.O.C.	No. 6, INNOVATION FIRST ROAD, HSINCHU SCIENCE PARK, BAOSHAN TOWNSHIP, HSINCHU COUNTY 30076, TAIWAN, R.O.C.

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1 Intended Use

HEPAVASE MA-96 (TMB) is an enzyme immunoassay kit for in vitro qualitative detection of IgM antibody to hepatitis A virus (Anti-HAV IgM) in human serum or plasma (heparin, EDTA or citrate).

2 Summary and Test Explanation

The hepatitis A virus (HAV) is a single-stranded RNA-containing virus without an envelope and with a diameter of 27 nm that belongs to the family of Picornaviridae (1-2). Hepatitis A - the most common form of acute viral hepatitis - is an infection of fecal-oral transmission produced in humans after an average incubation period of 28 days (range, 15-50 days). The illness caused by HAV infection typically has an abrupt onset of symptoms that can include fever, malaise, anorexia, nausea, abdominal discomfort, dark urine, and jaundice (2). Hepatitis A antigen can be detected in the feces only briefly before or at the onset of infection becoming generally undetectable during the late acute stage (3). The antibody specific to HAV during the acute phase of hepatitis A is the IgM type (Anti-HAV IgM), which decreases then being replaced by IgG type (Anti-HAV IgG) during early and late convalescence (4). Anti-HAV IgM usually disappears 3 to 4 months after the acute phase. An acute hepatitis A virus infection can be assumed if anti-HAV IgM antibody is detected (5). Anti-HAV IgM antibody develops only very rarely after vaccination (6). Assays to detect anti-HAV IgM antibodies are useful in distinguishing hepatitis A infection from other types of infections

HEPAVASE MA-96 (TMB) is a fast test for the qualitative detection of IgM antibody to Hepatitis A virus in serum or plasma (heparin, citrate or EDTA) specimens. This is an enzyme linked immunosorbent assay (ELISA) which utilizes Anti-human IgM on microtiter wells as solid phase and HAV Ag and peroxidase-conjugated Anti-HAV in liquid phase in an “IgM capture” principle to detect Anti-HAV IgM levels in serum or plasma.

Specimens with absorbance values **greater** than the Cutoff Value are considered **REACTIVE for Anti-HAV IgM.**

Specimens with absorbance values **less or equal** than the Cutoff Value are considered **NONREACTIVE for Anti-HAV IgM.**

The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above.

If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples of the patient.

3 Test Description

HEPAVASE MA-96 (TMB) is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immunosorbent assay) – based on the principle of "IgM capture". The solid phase of the microtiter plate is made of polystyrene wells coated with anti-human IgM, while peroxidase-conjugated Anti-HAV acts as liquid phase.

When a serum or plasma specimen containing Anti-HAV IgM is added to the Anti-human IgM-coated wells and incubated, IgM antibodies present in the specimen bind to the Anti-human IgM on the wells. After addition of an HAV Ag-containing solution and a solution containing Peroxidase-conjugated anti-HAV a further incubation takes place, during which (Anti-h IgM) • (Anti-HAV IgM) •(HAV Ag) • (Anti-HAV• Peroxidase) complex is formed on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. If Anti-HAV IgM is present in the specimen, after washing, the activity of peroxidase on the wells reflects the content of anti-HAV IgM in a specimen. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm[*1]

<u>The above described test principle is also shown as follows:</u>

A. Specimen (containing antibodies IgM Anti-HAV):

1. Plate (Anti-h IgM) + specimen (containing IgM Anti-HAV)
   → Plate (Anti-h IgM)· IgM Anti-HAV
2. Plate (Anti-h IgM)· IgM Anti-HAV + HAV + Anti-HAV·Peroxidase
   → Plate (Anti-h IgM)· IgM Anti-HAV·HAV·(Anti-HAV·HRPO) complex
3. Wash to remove the unbound materials.
4. plate (Anti-h IgM)· IgM Anti-HAV·HAV· (Anti-HAV·HRPO) complex
   + TMB solution → light blue to blue color.
5. Light blue to blue color + 2 N H_2SO_4 → light yellow to yellow color, measured at 450 nm with a selected reference wavelength within 620 to 690 nm[*1].

B. Specimen (without antibodies IgM Anti-HAV):

1. Plate (Anti-h IgM) + specimen (without IgM Anti-HAV) → Plate (Anti-h IgM)
2. Plate (Anti-h IgM) + HAV + Anti-HAV· Peroxidase → Plate (Anti-h IgM)
   ------ no complex will form
3. Wash to remove the unbound material.
4. Plate (Anti-h IgM) + TMB solution (colorless) → colorless
5. colorless + 2 N H_2SO_4 → colorless, measured at 450 nm with a selected reference wavelength within 620 to 690 nm[*1].

4 Description of Materials Provided & Product Code System

● **Storage Condition:** Item 1 - 8 on the following reagent table should be refrigerated at +2 to +8°C . Conc. Washing Solution D (20x) and Stop Solution 2 can be stored at + 2 to +30°C.

ITEMS	Material Code	Components	Description	Qt. per 96 tests	Qt. per 480 tests
(1)	3B040MPCM	Anti-h IgM Plate	Microtiter plate coated with purified antibody to human IgM.	1 plate	5 plates
(2)	3A071-M3/5	Anti-HAV · Peroxidase Solution	Anti-HAV · Peroxidase (horseradish) conjugate in buffer with protein stabilizers. Preservatives: 0.003 % gentamycin and 0.01 % thimerosal.	1bottle, 8 ml	3bottle, 8 ml
(3)	3A090-M3/5	CONTROL + Anti-HAV IgM Positive Control	Serum containing diluted Anti-HAV IgM in buffer with protein stabilizers. Preservatives: 0.003 % gentamycin and 0.01 % thimerosal.	1 bottle, 2.5 ml	2 bottles, 2.5 ml
(4)	3A115 3A115S	Specimen Diluent	Protein stabilizer in buffer. Preservatives: 0.003 % gentamycin and 0.01 % thimerosal.	1 bottle, 12 ml	5 bottles, 12 ml
(5)	3A118-V3/5	Hepatitis A Virus Solution	Hepatitis A virus antigen in buffer and protein stabilizer. Preservatives: 0.003 % gentamycin and 0.01 % thimerosal.	1 bottle, 8 ml	3 bottles, 8 ml
(6)	3A110-M3/5	CONTROL - Anti-HAV IgM Negative Control	Protein stabilizer in buffer. Preservatives: 0.003 % gentamycin and 0.01 % thimerosal.	1 bottle, 2.5 ml	2 bottles, 2.5 ml
(7)	3B135TMB-A 3B145TMB-A	TMB Substrate Solution A	3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle, 12 ml	3 bottles, 12 ml
(8)	3B140TMB-B 3B150TMB-B	TMB Substrate Solution B	Citric acid buffer containing H_2O_2.	1 bottle, 12 ml	3 bottles, 12 ml
(9)	3B112PBS3/5	Conc. Washing Solution D (20X)	Phosphate buffer with tween-20.	1 botlle 58 ml	5 botlles, 58 ml
(10)	3B155SACID2N 3B155SACID2N5	Stop Solution 2	2 N H_2SO_4	1 bottle 12 ml	5 bottles, 12 ml

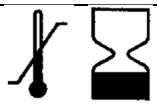
● ACCESSORIES: (provided as needed)

ITEMS	Material Code	Components
(11)	2P951001	Adhesive Slips
(12)	2P505001	Absorbent Pads
(13)	2P403001	Black Cover

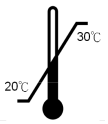
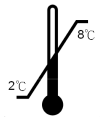
● OTHER MATERIALS AND DEVICES REQUIRED, BUT NOT PROVIDED

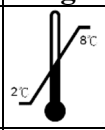
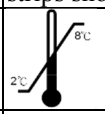
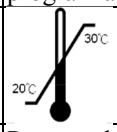
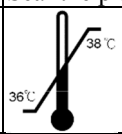
ITEMS	Components
(1)	5 µl, 50 µl, 100µl and 1.0 ml micropipettes and tips are needed
(2)	100 ml of 0.15 M Normal Saline.
(3)	Incubator or waterbath with temperature control at +37 °C.
(4)	Tubes for specimen dilution.
(5)	Plate washing equipment.
(6)	ELISA Microwell Reader: Dual wavelength 450 nm with 620-690 nm as reference wavelength[*1], bandwidth 10 nm.
(7)	Purified water: distilled or deionized water.
(8)	Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

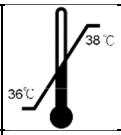
4.1 Storage Conditions and Stability of Kit and Components



Kit/Components	Storage condition	State	Stability
HEPAVASE MA-96 (TMB) KIT	+2～+8 °C	Original	18 months
		Once open	1 month
Anti-HAV IgM Positive Control	+2～+8 °C	Original	18 months
		Once open	1 month
Anti-HAV IgM Negative Control	+2～+8 °C	Original	18 months
		Once open	1 month
Hepatitis A Virus Solution	+2～+8 °C	Original	18 months
		Once open	1 month
Specimen Diluent	+2～+8 °C	Original	18 months
		Once open	1 month
Anti-h IgM Plate	+2～+8 °C	Original	18 months
		Once open	2 month
Anti-HAV·Peroxidase Solution	+2～+8 °C	Original	18 months
		Once open	1 month
Conc. Washing Solution D (20X)	Room temp.	Original	24 months
		Once open	1 month
20X Diluted Washing Solution	Room temp.	Diluted	2 days
	+2～+8 °C	Diluted	1 week
TMB Substrate Solution A	+2～+8 °C	Original	24 months
		Once open	1 month
TMB Substrate Solution B	+2～+8 °C	Original	24 months
		Once open	1 month
TMB substrate mixture	Room temp.	Mixture	6 hours
Stop Solution 2	Room temp.	Original	24 months
		Once open	1 month

5	**Instruction for Use**
5.1	**⚠Warnings**
5.1.1	This reagent kit is for professional use only.
5.1.2	IVD This reagent kit is for ***in vitro*** diagnostic use only.
5.1.3	 Bring all kit reagents and samples to room temperature (+20 to +30 °C) and mix gently before use.
5.1.4	 Do not use reagent beyond its expiration date.
5.1.5	Do not interchange reagents between different lots.
5.1.6	Do not pipette in the mouth.
5.1.7	Do not smoke or eat in areas where specimens or reagents are handled.
5.1.8	The positive control, HAV solution, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They shall be used and discarded according to the user's laboratory safety procedures. Such safety procedures probably shall include wearing protective gloves and avoiding aerosols generation.
5.1.9	Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5 % sodium hypochlorite or treated in accordance with the laboratory's practice for potential bio-hazard control.
5.1.10	**Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with your treatment practice of potential bio-hazardous waste or treated as follows:** Both liquid and solid waste should be autoclaved maintaining +121 °C for at least 30 minutes. Solid waste can also be incinerated. Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1 %. Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
5.1.11 	2 N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2 N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.
5.1.12 	TMB substrate solution A contains organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.
5.2	**Specimen Collection and Preparation for Analysis**
5.2.1	No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.
5.2.2	Either serum or plasma can be used with this diagnostic kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
5.2.3	 Specimens must be stored at +2 to +8 °C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20 °C. Storage in self-defrosting freezer is not recommended.
5.2.4	Frozen specimens must be thoroughly thawed and mixed homogenously before test.
5.2.5	Avoid multiple freeze-thaw procedures
5.2.6	
⚠**WARNING**	1. The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity. 2. Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.

5.3	**Reagents storage**
5.3.1	 The kit must be stored at +2 to +8 °C. Do not freeze.
5.3.2	Strips of the plate should be used within 2 months after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.
5.3.3	 Return reagents to +2 to +8 °C immediately after use.
5.3.4	Conc. Washing Solution D (20X) should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a +37 °C water bath till the crystal is dissolved.
5.4	**Plate washing procedure**
5.4.1	Preparation of washing solution: Dilute Conc. Washing Solution D (20X) with distilled or de-ionized water to 1:20 dilution. Do not use tap water.
5.4.2	Plate washing: 1. Plate washing: (a) For plate washer with overflow aspirating function: 6 cycles with at least 0.5 ml washing buffer per well per cycle. or (b) For plate washer without overflow aspirating function: 8 cycles with at least 0.35 ml washing buffer per well per cycle. 2. Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.
5.4.3	Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.
⚠ **WARNING**	Improper washing will cause false results.
5.5	**Test procedure**
	Assay process can be performed by an automatic EIA micro-plate immuno-analyzer. Please set up the program according to the following test procedure.
5.5.1	 Bring all reagents and specimens to room temperature (+20 to +30 °C) before assay. Adjust water bath or incubator to +37 ± 1 °C.
5.5.2	Prepare the needed number of wells, including two wells for blanks, three wells for Negative Control, two wells for Positive Control, and one well for each specimen. Reserve 2 wells for Blanks. Add **100 µl** of Negative Control to each of three wells, **100 µl** of Positive Control to each of the two wells, and **100 µl** of Specimen Diluent to each of the other reaction wells for the test specimens.
5.5.3	Make 1：200 dilution of each specimen: Prepare the tubes for dilution as number of specimens. Add 1.0 ml of Saline Solution and 5 µl of each specimen to each tube, respectively and shake to mix.
5.5.4	Add **5 µl of each diluted specimen** to each well containing Specimen Diluent, respectively.
5.5.5	Gently tap the plate.
5.5.6	Seal the plate with an adhesive slip.
5.5.7	 Incubate the plate in incubator or water bath at +37 ± 1 °C for **one hour**.
5.5.8	At the end of the incubation period, remove and discard the Adhesive Slip and wash plate in accordance with “5.4. **Plate washing procedure**”.
5.5.9	Add **50 µl** of Hepatitis A Virus Antigen Solution and **50 µl** of Anti-HAV·Peroxidase Conjugate Solution into each reaction well except the Blanks. Apply new adhesive slip.

5.5.10	 Incubate the plate in incubator or water bath at +37 ± 1°C for **one hour**.
5.5.11	At the end of the incubation period, remove and discard the adhesive slip, wash the plate in accordance with "5.4. **Plate washing procedure**".
5.5.12	Choice one of the following two methods for color development: **NOTE:** TMB Substrate Solution A should be colorless to light blue, otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mixing. The mixture should be avoided from intense light.
	A. Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 µl of the mixture solution to each well including the two blank wells. B. Add 50 µl of TMB Substrate Solution A first, then add 50 µl of TMB Substrate Solution B into each well including the two blanks. Mix well gently.
5.5.13	Cover the plate with black cover and incubate at room temperature for 30 minutes.
5.5.14	Stop the reaction by adding 100 µl of Stop Solution 2 to each well including the blank.
5.5.15	Determine the absorbance of controls and test specimens within 15 minutes with a photometer at 450 nm with a selected reference wavelength within 620 to 690 nm[*1]. Use the blank well to blank the photomer. NOTE: The color of the blank should be colorless to light yellowish; otherwise, the test result is invalid. In this case the test must be repeated. Substrate blank : absorbance value must be less than 0.100.
5.6	**Calculation of Tested Results**
5.6.1	Calculation of the NCx (Mean Absorbance of Negative Control). Example: Sample No. Absorbance 1 0.080 2 0.085 3 0.079 NCx = (0.080+0.085 + 0.079) / 3 = 0.081 NCx must be ≤ 0.2, otherwise, the test is invalid.
5.6.2	Calculation of PCx (Mean Absorbance of Positive Control) Example: Sample No. Absorbance 1 1.223 2 1.205 PCx = (1.223 + 1.205) /2 = 1.214 PCx must be ≥ 0.5 , otherwise, the test is invalid.
5.6.3	Calculation of P-N Value P-N = PCx - NCx Example: P - N = 1.214 – 0,081 = 1,133 P - N Value must be ≥ 0.3, otherwise, the test is invalid.
5.6.4	Calculation of the Cutoff Value Cutoff Value = NCx + (PCx)/4 Example: Cutoff Value = 0.081+(1.214/4) = 0.385
5.6.5	Calculation of the Retest Range Retest Range = Cutoff Value ± 10 % Example: Cutoff Value = 0.385 Retest Range = (0.385 - 0.039) to (0.385 +0.039) = 0.346 to 0.424
5.7	**Validity of Test Runs**
5.7.1	NCx must be ≤ 0.2, otherwise, the test is invalid.
5.7.2	PCx must be ≥ 0.5, otherwise, the test is invalid.
5.7.3	P-N Value must be ≥ 0.3, otherwise, the test is invalid.
	NOTE: Negative Control: absorbance value must be less than or equal to 0.200 after subtracting the blank.

5.8. Interpretation Results

5.8.1	Specimens with absorbance values LOWER than the Cutoff Value are considered non-reactive for Anti-HAV IgM
5.8.2	Specimen with absorbance value GREATER than or EQUAL TO the Cutoff Value is considered reactive for Anti-HAV IgM.
5.8.3	If the data is within the Retest Range, the test must be repeated in duplicate and interpreted as above. If the retested absorbance still within the retest range, it is suggested to test follow-up-samples.

5.9 Troubleshooting

If the result cannot be reproduced, a preliminary troubleshooting should be performed by checking the possibilities listed below:

5.9.1	Improper washing procedure.
5.9.2	Contamination with positive specimens.
5.9.3	Wrong volume of sample, conjugate or substrates.
5.9.4	Contamination of well rim with conjugate.
5.9.5	Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not being mixed well before use.
5.9.6	Wrong incubation time or temperature.
5.9.7	Obstructed or partial obstructed washer aspirate/dispense head and needles.
5.9.8	Insufficient aspiration.

5.10 Limitations and Interferences

5.10.1	This reagent kit is to be used for un-pooled human serum or plasma samples only.
5.10.2	Non-repeatable reactive results may be obtained with any enzyme immunoassay kit, largely due to technical error either on the part of the operator or malfunction of apparatus used.
5.10.3	The reagent kit has not been validated for use with cadaveric samples.
5.10.4	Potential interfering substances: By addition tests the following results were obtained: 1. The anticoagulants heparin, citrate and EDTA had no effect on the test result. 2. Hemoglobin up to 8.0 mg / ml had no effect on the test result. 3. Bilirubin up to 0.3 mg / ml had no effect on the test result. 4. Triglyceride up to 5.0 mg / ml had no effect on the test result. 5. A hheumatoid factor high positive specimen exhibited a false positive result. 6. Pregnancy did not effect the test result.

5.11 Performance Characteristics

5.11.1 Diagnostic Sensitivity and Diagnostic Specificity

1. Specimens from hospitalized patients:

		GBC HEPAVASE MA-96 (TMB)		
Comparison assay		NON-REACTIVE	REACTIVE	Total
	NON-REACTIVE	1378	0	1378
	REACTIVE	4	188	192
	total	1382	188	1570

Diagnostic sensitivity = 100 % x 188/192 = 98 %

Diagnostic specificity = 100 % x 1378/1378=100 %

2. Patients with acute hepatitis B:

		GBC HEPAVASE MA-96 (TMB)		
Comparison assay		NON-REACTIVE	REACTIVE	Total
	NON-REACTIVE	51	0	51
	REACTIVE	0	0	0
	total	51	0	51

Conformity = 100 %

3. Hepatitis B patients in convalescent period:

		GBC HEPAVASE MA-96 (TMB)		
		NON-REACTIVE	REACTIVE	Total
Comparison assay	NON-REACTIVE	28	0	28
	REACTIVE	0	0	0
	total	28	0	28

Conformity = 100 %

4. Chronic hepatitis B carriers:

		GBC HEPAVASE MA-96 (TMB)		
		NON-REACTIVE	REACTIVE	Total
Comparison assay	NON-REACTIVE	107	0	107
	REACTIVE	0	0	0
	total	107	0	107

Conformity = 100 %

5. Auto-immune patients:

		GBC HEPAVASE MA-96 (TMB)		
		NON-REACTIVE	REACTIVE	Total
Comparison assay	NON-REACTIVE	20	0	20
	REACTIVE	0	0	0
	total	20	0	20

Conformity = 100 %

6. Patients with acute hepatitis A:

		GBC HEPAVASE MA-96 (TMB)		
		NON-REACTIVE	REACTIVE	Total
Comparison assay	NON-REACTIVE	0	0	0
	REACTIVE	0	24	0
	total	0	24	24

Diagnostic sensitivity = 100 %
Diagnostic specificity = 100 %

7. Patients with other viral infections:

		GBC HEPAVASE MA-96 (TMB)		
		NON-REACTIVE	REACTIVE	Total
Comparison assay	NON-REACTIVE	35	0	35
	REACTIVE	0	0	0
	total	35	0	35

Diagnostic specificity/Conformity = 100 %

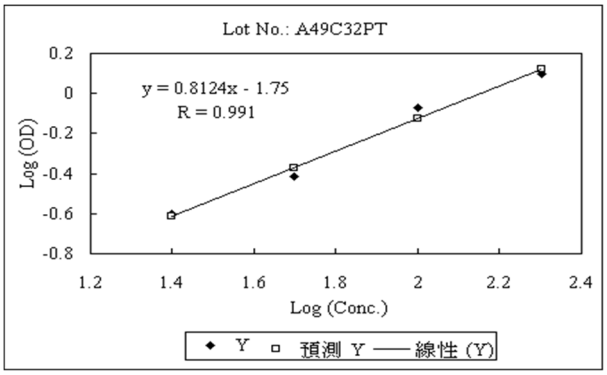
5.11.2

Analytical Sensitivity

Analytical sensitivity ≤ 100 GBU / ml

e.g. (Lot. No.: A49C32PT) Analytical sensitivity = 61.7 GBU / ml

Conc. (GBU / ml)	OD	Log (Conc.)	Log (OD)
200	1.256	2.30103	0.09898964
100	0.846	2	-0.07262964
50	0.385	1.69897	-0.41453927
25	0.250	1.39794001	-0.60205999
Cutoff	0.506	1.79003187	-0.29584948
Sensitivity **(GBU / ml)**	61.7	----------	----------



預測 = prediction

線性 = Linearity

Linear regression	
R	0.991195
R^2	0.982468
Adjusted R^2	0.973702
Standard error	0.051643
Number of observed value	4

5.11.3	**Precision**
	Intra-assay reproducibility: Intra-assay CV % < 15 %
	Inter-assay reproducibility: Inter-assay CV % < 20 %
5.11.4	**Traceability**
	Concentration of Anti-HAV IgM Positive Control = 800 ± 200 GBU / ml

5.12	Flow chart of the test procedure

Simplified procedure of HEPAVASE MA-96 (TMB)

Dilute 5 µl of each specimen with 1 ml saline solution. DO NOT DILUTE CONTROLS.

↓

Add 100 µl Controls (3 x NC, 2 x PC) and 100 µl specimen diluent into wells. Reserve 2 wells for Blanks.

↓

Add 5 µl of the diluted specimen into each of wells containing 100 µl specimen diluent, respectively. Seal with adhesive slip.

↓

Incubate at +37 ± 1 °C for one hour

↓

Wash the plate

↓

Add 50 µl of HAV Antigen Solution and 50 µl Anti-HAV·Peroxidase Conjugate Solution into each reaction well, except Blanks.

↓

Incubate at +37 ± 1 °C for one hour

↓

Wash the plate

(Choose one of the following two methods for color development)

↙ ↘

Mix equal volumes of TMB Substrate Solution A and B. Add 100 µl of the mixed solution to wells.

Add 50 µl of TMB Substrate Solution A to wells and then add 50 µl of TMB Substrate Solution B. Mix well, gently.

↘ ↙

Incubate at R.T. for 30 minutes.

↓

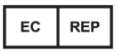
Add 100 µl of Stop Solution 2 into each well.

↓

Determine absorbance using 450 nm as reading wavelength with 620-690 nm reference wavelength[*1].

6	**Bibliography**

*1. The reference wavelength of spectrometer could be 620 nm to 690 nm. However, user should validate the spectrometer in combination with this kit before use.

	Symbols Key / Symbolschlüssel / Erklärung des Symbols / Interpretazione simboli / Clave dos Simbolos
	Manufacturer / Hersteller / Fabricante / Fabbricante / Fabricant / Fabricante
	In Vitro Diagnostic Medical Device / In-Vitro-Diagnostikum / Producto sanitario para diagnóstico in vitro / Dispositivo medico-diagnostico in vitro / Dispositif médical de diagnostic in vitro / Dispositivo médico para diagnóstico in vitro
	Batch code / Chargenbezeichnung / Código de lote / Codice del lotto / Code du lot / Código do lote
	Use By / Verwendbar bis / Fecha de caducidad / Utilizzare entro / Utiliser jusque / Prazo de validade
	Temperature limitation / Temperaturbegrenzung / Límite de temperatura / Limiti di temperatura / Limites de température / Limites de temperatura
	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / CE Marca / Marca CE
	Catalogue number / Bestellnummer / Número de catálogo / Numero di catalogo / Référence du catalogue / Referência de catálogo
	Consult Instructions for Use- / Gebrauchsanweisung beachten / Consulte las instrucciones de uso / Consultare le istruzioni per l'uso / Consulter les instructions d'utilisation / Consulte as instruções de utilização
	Caution,consult accompanying documents / Achtung, Begleitdokumente beachten / Atención,ver instrucciones de uso / Attenzione, vedere le istruzioni per l'uso / Attention voir notice d'instructions / Atenção, consulte a documentação incluída
	Authorized representative/ Bevollmzed repre Vertreter / repreprer / autoriss/ Rappresentante autorizzato/ Reprezentant autorizat/ Representante autorizado

End of the Instruction for Use

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Product Name	**GB HIV Ag-Ab COMB**		
IVD	**Product Code**	**4EAIC11/ 4EAIC13**	CE 0344
Intended Use	The GB HIV Ag-Ab COMB assay is a 4th generation solid phase Enzyme-Linked Immunosorbent assay using a mixture of antigens and antibodies for qualitative *in vitro* diagnostic screening in human serum or plasma of antibodies to HIV-1(group M and O), HIV-2 and HIV-1 p24 antigen.		

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1) Intended Use

The GB HIV Ag-Ab COMB assay is a 4th generation solid phase Enzyme-Linked Immunosorbent assay using a mixture of antigens and antibodies for the qualitative *in vitro* diagnostic screening in human serum or plasma of antibodies to HIV-1(group M and O), HIV-2 and HIV-1 p24 antigen.

2) Introduction

Acquired immune deficiency syndrome (AIDS) is a set of symptoms resulting from the incapacitation of the human immune system caused by the Human Immunodeficiency Virus (HIV). HIV infection may progress to a symptomatic phase that is characterized by opportunistic infections and that may cause death.

The etiological agent of AIDS, HIV, targets specific types of T cells causing Lymphopenia and affecting T cell mediated immunity. HIV is a member of a retrovirus family with two sub-families: HIV-1 and HIV-2. HIV-1 is more virulent and transmittable than HIV-2. HIV-1 is the cause of HIV infections globally, whereas HIV-2 is found predominantly in the countries of West Africa. As serological cross-reaction between HIV-1 and HIV-2 is highly variable and dependent on the tested sample, antigens for the specific detection of both HIV-1 and HIV-2 are included in the assay.

HIV is transmitted through sexual contact with infected persons, sharing needles and syringes with infected people and transfusion of contaminated blood. Enzyme-Linked Immunosorbent Assays (such as the GB HIV Ag-Ab COMB) are recommended for screening human blood and plasma for the presence of anti-HIV antibodies and HIV-1 p24 antigen. The presence of anti-HIV-1 and/or anti-HIV-2 in the blood indicates potential infection with HIV-1 and/or HIV-2 and consequently this blood should not be used for transfusion or for manufacture of injectable products.

3) Design Theory/ Brief Description of the Product

Antigens representing epitopes of HIV-1 gp41 and HIV-2 gp36 are coated onto microplate wells together with monoclonal antibodies against HIV-p24. Serum or plasma sample is added to the well and if antibodies specific for HIV-1 and/or HIV-2 (IgG, IgM or IgA) are present in the sample, stable complexes will be formed with the HIV antigens attached to the well. HIV-p24 antigen, if present will bind simultaneously to the antibodies in the well and to the detector antibodies present in the Sample Diluent. Non-reactive antibodies are removed by washing. Stable antigen-antibody complexes are identified through the successive addition of biotinylated antigens and horseradish peroxidase (HRP) conjugated streptavidin. These antibody-antigen complexes are quantified through the catalytic activity of horseradish peroxidase. Peroxidase substrate solution is added and is converted to a blue-coloured product. A positive sample generates a dark blue colour while faint blue color or colorless wells indicate a negative sample. Upon adding stopping solution, the colour of the solution will change from blue to yellow. Optical Density (OD) is measured with a spectrophotometer (ELISA reader) at 450 nm with reference wavelength at 620-690 nm and is in proportion to the amount of anti-HIV1/2-antibodies and HIV-P24 present in the sample.

4) Description of Provided Materials & Product Code System

● Item 1–10 on the following reagent table should be refrigerated at +2 to +8°C.
Conc.Washing Solution E (25X) and stop solution 1 can be stored at +2 to +30°C.

ITEMS	Material Code	ID No.	Components	Description	Qt. per 96 tests	Qt. per 480 tests
(1)	3EAIC011	3EAIC011	MICROPLATE	**Microplate** 96-well microtiter plate coated with HIV-1 gp41, HIV-2 gp36 antigen and monoclonal antibody against HIV-1 p24.	1 plate	5 plates
(2)	3EAIC021/3	3EAIC021/3	CONJ 1	**HIV COMB Conjugate 1** Phosphate buffer containing HIV-1 and HIV-2 biotinylated antigens and blue dye. Preservative: 0.05% Proclin™300. Ready to use.	1 bottle, 25 ml	1bottle, 120 ml
(3)	3EAIC031/3	3EAIC031/3	CONJ 2 101X	**101X Concentrated Conjugate 2** Streptavidin-horseradish peroxidase. To be diluted 1:101 in conjugate diluent 2 before use.	1 bottle, 0.25 ml	1bottle, 1.3 ml
(4)	3EAIC041/3	3EAIC041/3	CONJ 2 DIL	**HIV COMB Conjugate Diluent 2** Phosphate buffer containing protein stabilizers and yellow dye. Preservative: 0.05% Proclin™300.	1 bottle, 25 ml	1bottle, 120 ml
(5)	3EAIC051/3	3EAIC051/3	CONTROL + HIV-1	**Anti-HIV-1 Positive Control** Inactivated rabbit serum reactive for anti-HIV1. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin. Ready to use.	1 bottle, 1.5 ml	1bottle, 3 ml
(6)	3EAIC061/3	3EAIC061/3	CONTROL + HIV-2	**Anti-HIV-2 Positive Control** Inactivated rabbit serum reactive for anti-HIV2. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin. Ready to use.	1bottle, 1.5 ml	1bottle, 3 ml
(7)	3EAIC071/3	3EAIC071/3	CONTROL + HIV-1 P24	**HIV-1 p24 Antigen Positive Control** Diluted human serum spiked with recombinant HIV-1 p24. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin. Ready to use.	1 bottle, 1.5 ml	1bottle, 3 ml
(8)	3EAIC081/3	3EAIC081/3	CONTROL -	**HIV COMB Negative Control** Normal human serum non-reactive for HBsAg, anti-HCV, anti-HIV-1 and 2 and HIV-1 p24. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin. Ready to use.	1 bottle, 1.5 ml	1bottle, 3 ml
(9)	3EAIC091/3	3EAIC091/3	SPE DIL	**HIV COMB Specimen Diluent** Tris- buffer contains biotinylated monoclonal antibodies against HIV-1 p24 and red dye. Preservative: 0.05% Proclin™300. Ready to use.	1 bottle, 12ml	1bottle, 60ml
(10)	3B135TMB-A/ 3B145TMB-A	30007-3A0/ 30005-3A0	SUBS TMB A	**TMB Substrate Solution A** Contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle, 12ml/bottle	1 bottle, 35ml
(11)	3B140TMB-B/ 3B150TMB-B	30007-3B0/ 30005-3B0	SUBS TMB B	**TMB Substrate Solution B** Acetic acid buffer with Urea Hydrogen Peroxidase.	1 bottle, 12ml/bottle	1 bottle, 35ml
(12)	3B155SACID1N/5N	30001-370/ 30008-370	SOLN STOP	**Stop Solution 1** 1N Sulfuric acid solution. Ready to use.	1 bottle, 12ml	1 bottle, 50ml
(13)	3EAIC101/3	3EAIC101/3	WASH BUF 25X	**Conc.Washing Solution E(25X)** A Concentrated Phosphate buffer with Tween-20.	1 bottle, 58ml	1 bottle, 250ml

● **ACCESSORIES: (provided as needed)**

ITEMS	Material Code	Components
(11)	2P951001	Adhesive slips
(12)	2P505001	Absorbent pads
(13)	2P403001	Black cover

● **OTHER MATERIALS REQUIRED, BUT NOT PROVIDED**

ITEMS	Components
(1)	50 µl, 100 µl and 200 µl, 1 ml micropipettes and tips are needed.
(2)	Water-bath or incubator
(3)	Tubes for specimen dilution.
(4)	Plate washing equipment.
(5)	ELISA Microwell Reader: Dual wavelength 450 nm with 620-690 nm as reference wavelength[*4], bandwidth 10 nm.
(6)	Purified water: distilled or deionized water.
(7)	Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

4.1) Storage Conditions and Stability of the kit and components

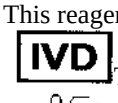
Kit/components	Storage condition	State	Stability
GB HIV Ag-Ab COMB KIT	+2 to +8°C	Original	18 months
		Once open	1 month
HIV Ag-Ab Plate	+2 to +8°C	Original	18 months
		Once open	1 months
HIV COMB Conjugate 1	+2 to +8°C	Original	18 months
		Once open	1 months
101XConcentrated Conjugate 2	+2 to +8°C	Original	18 months
		Once open	1 months
HIV COMB Conjugate Diluent 2	+2 to +8°C	Original	18 months
		Once open	1 month
Diluted Conjugate 2	Room temp.	Diluted	6 hours
	+2 to +8°C	Diluted	2 days
Anti-HIV-1 Positive Control	+2 to +8°C	Original	18 months
		Once open	1 month
Anti-HIV-2 Positive Control	+2 to +8°C	Original	18 months
		Once open	1 month
HIV-1 P24 Antigen Positive Control	+2 to +8°C	Original	18 months
		Once open	1 month
HIV COMB Negative Control	+2 to +8°C	Original	18 months
		Once open	1 month
HIV COMB Specimen Diluent	+2 to +8°C	Original	18 months
		Once open	1 month
Conc.Washing Solution E (25X)	Room temp.	Original	18months
		Once open	1 month
Diluted Washing Solution	Room temp.	Diluted	2 days
	+2 to +8°C	Diluted	1 week
TMB Substrate Solution A	+2 to +8°C	Original	24 months
		Once open	1 month

Kit/components	Storage condition	State	Stability
TMB Substrate Solution B	+2 to +8°C	Original	24 months
		Once open	1 month
Stop Solution 1	Room temp.	Original	24 months
		Once open	1 month

5) Instructions for Use

5.1) Warnings: 

5.1.1) This reagent kit is for professional use only.

5.1.2)  This reagent kit is for *in vitro* diagnosis only.

5.1.3)  Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix carefully before use.

5.1.4)  Do not use reagent past its expiration date.

5.1.5) Do not interchange reagents between different lots.

5.1.6) Do not put pipette in mouth.

5.1.7) Do not smoke or eat in areas where specimens or reagents are handled.

5.1.8) All kit components and specimens should be regarded as potential hazards to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols.

5.1.9) Potential infectious specimens and non-acid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with your practice for potential bio-hazard control.

5.1.10) Prior to disposition specimens, used kit reagents or possibly infectious materials as general waste; it should be treated in accordance with your treatment practice of potential bio-hazardous waste or treated as follows:
Both liquid and solid waste should be autoclaved at 121°C for at least 30 minutes.
Solid waste can also be incinerated.
Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%.
Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.

5.1.11)  Stop solution is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the stop solution with skin and mucous membranes. In case of contact, flush immediately with abundant amounts of water.
In case of inhalation, find fresh air immediately and seek medical advice in case of pain.

5.1.12)  TMB Substrate Soln. A contains organic solvent, which is flammable. TMB Substrate Soln. A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.

5.1.13)  Although all human sourced material, such as Anti-HIV Positive Control, HIV Antigen Positive Control CONTROL + and Negative Control CONTROL −, are tested free from HBsAg and Anti-HCV and inactivated at 56°C for one hour, the reagent should still be handled as potential infectious material. *[5]

5.2) Specimen Collection and Storage

5.2.1) Either serum or plasma can be used with this diagnostic kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.

5.2.2) Specimens must be stored at +2 to +8°C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below –20°C. Storage in self-defrosting freezer is not recommended.

5.2.3) Specimens should not be stored for periods longer than 10 days at +2 to +25°C.

5.2.4) Anticoagulants that may be used for specimen collection are: EDTA, Sodium Heparin, and Sodium Citrate.

5.2.5) Frozen specimens must be thoroughly thawed and mixed homogenously before test.

5.2.6) Avoid multiple freeze-thaw procedures.

5.2.7) **WARNING**

Incompletely coagulated sera and microbial-contaminated specimens should not be used.

5.3) Reagents Storage

5.3.1) The kit must be stored at +2 to +8°C. Do not freeze.

5.3.2) Strips of the plate should be used within one month once the original aluminum foil bag is opened. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.

5.3.3) Return reagents to +2 to +8°C immediately after use.

5.3.4) Conc.Washing Solution E (25X) can be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in 37°C water bath till crystal dissolved.

5.4) Plate Washing Procedure

5.4.1) Preparation of washing solution:

Dilute Conc.Washing Solution E (25X) with distilled or de-ionized water to 1:25 dilution. Do not use tap water.

5.4.2) Plate washing:

For plate washer with overflow aspirating function: 3-5 cycles with at least 0.4 ml wash buffer per well per wash and soaking at least 10 seconds.

5.4.3) Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.

WARNING

Improper washing will cause false results.

5.5) Test Procedure

5.5.1) Bring all reagents and specimens to room temperature (+20 to +30°C) before assay. Adjust water bath or incubator to 37 ± 1 °C.

5.5.2) Reserve one well for blank.

Do not add any specimen diluent、specimen or conjugates into the well for blank.

5.5.3) Take the necessary amount of test-strips and return non-used strips in the aluminum foil bag.

5.5.4) Add 100 µl of Specimen Diluent to each well except the well for blank.

5.5.5)Add 100ul of Specimen, 100ul of Negative and Positive controls in duplicate, mix well by pipetting up and down for homogenization. Pipette gently avoiding overflowing and contaminating adjacent wells.

NOTE: Use a new pipette tip after each sampling to avoid cross-contamination.

5.5.6) Seal the Plate with an Adhesive Slip.

5.5.7) Incubate the plate in a 37 ±1 °C water bath or circulative incubator for 60 minutes.

NOTE: Do not stack plates.

5.5.8) At the end of the incubation period, remove carefully the adhesive slip and discard.

5.5.9) Wash the plate **5** cycles according to section **5.4) Plate Washing Procedure.**

5.5.10) Add 200ul of ready to use **Conjugate 1** solution to the wells except the blank well.

5.5.11) Seal the Plate with an Adhesive Slip.

5.5.12) Incubate the plate in a 37 ±1 °C water bath or circulative incubator for 30 minutes.

NOTE: Do not stack plates.

5.5.13) At the end of the incubation period, remove carefully the adhesive slip and discard.

5.5.14) Wash the plate **3** cycles according to section**5.4) Plate Washing Procedure.**

5.5.15) Preparationof **Diluted Conjugate 2**

5.5.15.1) Use only clean container to avoid contamination.

5.5.15.2) Prepare **Diluted Conjugate 2**by making 1:100 dilution of 101x ConcentratedConjugate 2 with conjugate diluent 2, or following Conjugate Preparation Chart below. Swirl gently to mix thoroughly and avoid foaming.

5.5.15.3) Excess diluted conjugate solution should be discarded after use.

Diluted Conjugate 2 Preparation Chart:

Number of Wells used	Volume of ***ConjugateDiluent2*** needed (ml)	Volume of ***101X ConcentratedConjugate 2*** needed (µl)
8	2.0	20
16	4.0	40
24	6.0	60
32	8.0	80
40	10.0	100
48	12.0	120
56	14.0	140
64	16.0	160
72–80	18.0	180
81–96	25.0	250

5.5.16) Add 200µl of the Diluted Conjugate2 in each wellexcept the blank well.
5.5.17) Seal the plate with an Adhesive Slip.
5.5.18)  Incubate the Plate in a 37±1 °C water bath or circulative incubator for 30 minutes.
5.5.19) Repeat step 5.5.8) and 5.5.9)
5.5.20)  Select one of the following methods for color development:

A. Mix equal volumes of TMB Substrate Soln. A and B in a clean container immediately prior to use. Add 100 µl of the mixture solution to each well including the blank.
B. Add 50 µl of TMB Substrate Soln. A first, and then add 50 µl of TMB Substrate Soln. B into each well including the blank. Carefully mix well.

 **NOTE: TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. TheTMB Substrate Solution A and Bshould be avoided from intense light.**

5.5.21) Cover the plate with a black cover and incubate at room temperature(+20 to +30°C) for 30 minutes.
5.5.22) Stop the reaction by adding 100µl of stop solution 1 to each well including the blank.
5.5.23) Determine the absorbance of Controls and test specimens within 30 minutes, measured at 450nm with a selected reference wavelength within 620 to 690nm[*4].
Use the blank well to blank the spectrophotometer.

 **NOTE: The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid. Substrate blank: absorbance value must be less than 0.100.**

5.6) Calculation of Test Results

5.6.1) Calculation of the NCx (Mean Absorbance of Negative Control)
Example:

Sample No.	Absorbance
1	0.021
2	0.025

NCx = (0.021+0.025) / 2 = 0.023

 **NCx,must be ≤ 0.200, otherwise the test is invalid.**

5.6.2) Calculation of Anti-HIV-1PCx (Mean Absorbance of Anti-HIV-1 Positive Control)
Example:

Sample No.	Absorbance
1	1.545
2	1.239

Ab-PCx = (1.545 + 1.239) /2 = 1.392

⚠ **Anti-HIV-1 PCx must be ≥0.600, otherwise the test is invalid.**

5.6.3) Calculation of Anti-HIV-2 PCx (Mean Absorbance of Anti-HIV-2 Positive Control)

Example:

Sample No.	Absorbance
1	1.459
2	1.343

Ab-PCx = (1.459 + 1.343) /2 =1.401

⚠ **Anti-HIV-2PCx must be ≥ 0.600, otherwise the test is invalid.**

5.6.4) Calculation of HIV-1 P24 Ag PCx (Mean Absorbance of HIV-1 P24 Antigen Positive Control)

Example:

Sample No.	Absorbance
1	2.223
2	2.172

Ag-PCx = (2.223 + 2.172) /2 = 2.198

⚠ **HIV-1 P24 Ag PCx must be ≧ 0.600, otherwise the test is invalid.**

5.6.5) Calculation of the P-N Value

P – N = PCx - NCx

Example:

P-N = 2.198 – 0.023 = 2.175

⚠ **P – N Value must be ≧ 0.400, otherwise the test is invalid.**

5.6.6) Calculation of the **Cutoff Value**

Cutoff Value = NCx + 0.170

Example:

Cutoff Value = 0.023+0.170= 0.193

5.6.7) Calculate the cut-off index of the specimens

Cutoff Index

=Sample OD Value / Cutoff Value

Example:

Sample Value is 0.854

Cutoff Index = 0.854/0.193 = 4.424

5.7) ⚠ **Validity of the Test Run**

5.7.1) **NCx must be ≤ 0.200, otherwise the test is invalid.**

5.7.2) **Anti-HIV-1 PCx must be ≥0.600, otherwise the test is invalid.**

5.7.3) **Anti-HIV-2 PCx must be ≥0.600, otherwise the test is invalid.**

5.7.4) **HIV-1 P24 Ag PCx must be ≥ 0.600, otherwise the test is invalid.**

5.7.5)**P-N Values for Anti-HIV-1 PCx , Anti-HIV-2 PCx , and HIV-1 P24 Ag PCxmust be ≥ 0.400, otherwise the test is invalid.**

⚠ **NOTE:** Negative Control: absorbance value must be less than or equal to 0.200 after subtracting the blank.

5.8) Result Interpretation

5.8.1) Specimens with **CUTOFF INDEX < 1.000** are considered to be**NON-REACTIVE** by the criteria of this immunoassay, and may be considered negative for antibodies to HIV1+2 and HIV-1 P24. Further testing is not required.

5.8.2) Specimens with **CUTOFF INDEX ≥ 1.000** are considered as initially **REACTIVE**for HIV-1 and/or HIV-2 antibodies or HIV-1 P24. They should be **RETESTED** in duplicate. These specimens (using the original specimen)

should be re-tested in duplicate before final confirmation of the result.

5.8.3) Initially reactive specimens, which do not react in either of the duplicate repeat tests, are considered negative for antibodies of HIV1+2 and HIV-1 p24. Further testing is not required.

5.8.4) If one of both retested **CUTOFF INDEX** is ≥ **1.000**, the specimen is considered repeatedly**REACTIVE**. Specimens that have been found repeatedly reactive are interpreted to be **REACTIVE** for the presence of antibodies to HIV-1 and/or HIV-2 or HIV-1 p24. In most settings it is appropriate to investigate repeatedly reactive specimens by additional, more specific tests.

5.9) Troubleshooting

If the result cannot be reproduced, please do your own preliminary troubleshooting by checking the possibilities listed below:

5.9.1) Improper washing procedure.
5.9.2) Contaminated with positive specimen.
5.9.3) Add wrong volume of sample, conjugate or substrates.
5.9.4) The well rim is contaminated with conjugate.
5.9.5) Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not being mixed well before use.
5.9.6) Wrong incubation time or temperature.
5.9.7) Obstructed or partial obstructed washer aspirate/dispense head and needles.
5.9.8) Insufficient aspiration.

5.10) Limitations and Interferences

5.10.1) Strictadherence to the protocol is necessary to obtain reliable test results. Accurate sample and reagent pipetting and timing of washing and incubation should be respected.
5.10.2) This reagent kit is to be used for un-pooled human serum or plasma only.
5.10.3) The reagent kit has not been validated for use with cadaveric samples.
5.10.4) Specimens with very low level of Anti-HIV and/or HIV Ag may not consistently repeat reactive. In this case, it is recommended to test follow-up samples.
5.10.5) Anti-HIV negative result does not preclude the possibility of infection with HIV.
5.10.6) Non-repeatable false positive results may occur due to non-specific binding of the sample and conjugate to the wall of the well(s).
5.10.7) Potential Interfering Substances: there is no significant influence on GB HIV Ag-Ab COMB.
5.10.8)As with all in vitro diagnostic tests, a definitive clinical diagnosis should not be made based only on the results of a single test. A complete evaluation by a physician is needed for a final diagnosis.

6) Performance Characteristics

6.1 Specificity

6.1.1 Diagnostic Specificity (Normal Human Sera)

Specificity has been evaluated by testing 2 types of specimen samples:
Type 1: Blood Donor Samples
Type 2: Hospitalized Patient Samples

The results are shown in the following table:

Type#	#Samples Tested	Initially Reactive	Repeated Reactive	% Specificity
1	5126	7	5	99.9%
2	208	2	2	99.04%
Total	**5334**	**9**	**7**	**99.9%**

6.1.2 Specificity Interference

70 potential interfering samples and samples from pregnant women, including samples from multipara women were tested: Anti-HTLV I/II, Anti-HCV,Anti-HBc, Anti-HBs, Anti-HEV, Pregnant Women. None of the 70 potentially interfering specimens tested showed a S/CO higher than 1.

35 potentially cross-reactive samples were also tested: Rheumatoid Factor, CMV, EBV, Malaria, Syphillis, Herpes. 12 potentially interfering samples were also tested: Hemoglobin, Bilirubin, ANA, HAMA, GAMMA-IgG, HBsAg, HCV Core Ag, TG.

Parameter	#Tested	Reactive	% Specificity
Anti-HTLV I/II	10	0	100%
Anti-HCV	10	0	100%
Anti-HBc	15	0	100%
Anti-HBs	15	0	100%
Anti-HEV	10	0	100%
Pregnant	7	0	100%
Multipara Samples	3	0	100%
Rheum. Factor	10	0	100%
CMV	5	1	80%
EBV	5	1	80%
Malaria	5	0	100%
Syphillis	5	0	100%
Herpes	5	0	100%
Total	**105**	**2**	**98%**

12 potentially interfering samples were also tested: Hemoglobin(3.4 g/dl), Bilirubin(0.4 mg/ml), ANA (25%), HAMA(17 ng/ml), GAMMA-IgG(6 mg/ml), HBsAg(0.5 IU/ml), HCV Core Ag(15.57 ug/ml), TG(300 mg/ml). Results for these show that GB HIV Ag-Ab COMB Assay is not affected by these potentially interfering substances.

6.2 Sensitivity

6.2.1 Positive Samples

HIV Ag/Ab positive samples were tested for sensitivity using GB HIV Ag-Ab COMB Kit. The number of specimen tested were: HIV-1 Ab positive specimen (n=310), HIV-1 Ab positive specimen, non-B subtype (n=40), HIV Ab/Ag positive specimen (n=50), HIV-2 Ab positive specimen (n=60).

Specimen Type	Reactive	Non-Reactive	Sensitivity
HIV-1 Ab Positive Specimen	310	0	100%
HIV-1 Ab Positive Specimen, non-B subtype	44	0	100%
HIV Ab/Ag Positive Specimen	50	0	100%
HIV-2 Ab Positive Specimen	100	0	100%
Total	**504**	**0**	**100%**

6.2.2 Performance samples

Commercial samples from NIBSC were tested for immune-reactivity.All samples are detected with the GB HIV Ag-Ab COMB kit.

NIBSC reference samples	NIBSC Code	OD 450/650nm	CO	OD/CO	Result
NIBSC BWS for anti HIV-1	99/750	1.989	0.268	7.4	POS
NIBSC 1 in 5 BWS for anti HIV	99/710	1.204	0.268	4.5	POS
NIBSC Monitor Sample for anti HIV-2	99/674	3.596	0.268	13.3	POS
NIBSC, ref panel 02/210, HIV-1/subt A	02/210	3.727	0.228	16.4	POS
NIBSC, ref panel 02/210, HIV-1/subt B	02/210	3.718	0.228	16.3	POS
NIBSC, ref panel 02/210, HIV-1/subt C	02/210	3.970	0.228	17.5	POS
NIBSC, ref panel 02/210, HIV-1/subt E	02/210	4.000	0.228	17.6	POS
NIBSC, ref panel 02/210, HIV-1/group O	02/210	3.898	0.228	17.1	POS
NIBSC, ref panel 02/210, anti HIV-2	02/210	3.808	0.228	16.7	POS
NIBSC, ref panel 02/210, diluent control	02/210	0.049	0.228	0.2	NEG

6.2.3 Seroconversion panels

20 seroconversion panels from commercial supplierSeraCare were tested with the GB HIV Ag-Ab COMB kit. Results were compared against Enzygnost Anti-HIV 1/2 Plus and Genscreen HIV-1/2 v2 as 3rd Generation assays, Vironostika HIV Uniform II Ag/Ab (CE Marked), Vironnostika HIV Ag/Ab(CE Marked), and Vidas HIV Duo Quick as 4th Generation assays, and INNOTEST HIV Ag as Anitgen Reference Test.

Panel ID	GB HIV Ag-Ab COMB	Vironostika HIV Uniform II Ag/Ab Or Vironnostika HIV Ag/Ab** Or Vidas HIV Duo Quick*** (4th Gen Reference Test)
PRB945	4/6	3/6**
PRB946	2/4	0/4**
PRB947	3/4	3/4
PRB949	2/4	1/4**
PRB950	3/4	1/4
PRB955	4/5	3/5
PRB956	2/5	0/5
PRB958	4/6	2/6
PRB962	2/6	2/6**
PRB963	2/7	1/7**
PRB964	1/6	0/6**
PRB967	3/6	3/6**
PRB969	4/10	3/10**
PRB970	4/4	3/4**
PRB973	2/4	1/4**
PRB974	3/4	1/4**
PRB976	4/4	2/4**
PRB977	2/4	2/4**
PRB978	1/7	1/7**
PRB961	2/9	2/9***
Total Score	**54/109**	**34/109**

6.2.4 Analytical sensitivity

6.2.4.1 Analytical sensitivity for anti-HIV-1 antibody

The analytical sensitivity for anti-HIV-1 antibody using GB HIV Ag-Ab COMB kit was assessed by testing commercially available PRB109(M)Anti-HIV-1.

Item		**ODx**		**COI**	
Kit brand		GB	Adaltis	GB	Adaltis
Kit lot / Name		D56C01PT	16315	D56C01PT	16315
PRB109(M) Anti-HIV-1 low-titer set	#1	3.039	2.372	11.8	9.8
	#2	3.925	3.772	15.2	15.6
	#3	2.229	1.215	8.6	5.0
	#4	3.359	2.189	13.0	9.1
	#8	0.056	0.062	0.2	0.3

Item		ODx		COI	
Kit brand		GB	Adaltis	GB	Adaltis
Kit lot / Name		D56C01PT	16315	D56C01PT	16315
PRB109(M) Anti-HIV-1 low-titer set	#10	3.706	3.102	14.3	12.9
	#11	3.896	3.472	15.1	14.4
	#12	3.399	3.230	13.1	13.4
	#13	3.320	2.137	12.8	8.9
	#14	3.953	3.663	15.3	15.2
	#15	3.763	3.825	14.6	15.9
	#16	3.875	3.881	15.0	16.1
	#17	3.894	3.630	15.1	15.1
	#19	3.852	3.871	14.9	16.1
	#20	2.219	2.397	8.6	9.9
Conclusion		The analytical sensitivity of GB HIV Ag-Ab COMB in detecting anti-HIV-1 antibody is comparative to the Adaltis kit.			

6.2.4.2 Analytical sensitivity for anti-HIV-2 antibody

The analytical sensitivity for anti-HIV-2 antibody using GB HIV Ag-Ab COMB kit was assessed by testing commercially available NIBSC 99/674 Anti-HIV-2

Panel	Dilution Fold	ODx		COI	
		GB	Adaltis	GB	Adaltis
NIBSC 99/674 Anti-HIV-2	2X	3.895	0.436	15.1	1.8
	4X	2.739	0.188	10.6	0.8
	8X	1.374	0.122	5.3	0.5
	16X	0.860	0.090	3.3	0.4
	32X	0.455	0.075	1.8	0.3
	64X	0.285	0.073	1.1	0.3
	128X	0.178	0.067	0.7	0.3
Conclusion		The analytical sensitivity of GB HIV Ag-Ab COMB in detecting anti-HIV-2 antibody is superior to the Adaltis kit.			

6.2.4.3 Analytical sensitivity for HIV-1 p24 antigen

The analytical sensitivity for HIV-1 p24 antigen using GB HIV Ag-Ab COMB kit was assessed by testing commercially available NIBSC International Standard for HIV-1 p24 antigen, Cat#90/636).

The tables below show that GB HIV Ag-Ab COMB kit can detect 0.391 IU/mL.

Conc. IU/ml NIBSC 90/636	GB HIV Ag-Ab COMB S/co	Genscreen Ultra HIV Ag/Ab S/co
3.125	5.64	2.03
1.563	3.17	1.10
0.781	1.73	0.74
0.391	1.12	0.61
0.195	0.88	0.47
0.098	0.53	NT

6.2.5 Spectrum of p24 Antigen detection (Cell Culture)

50 cell culture supernatants covering different genotypes were assayed for presence of HIV-p24 antigen: HIV-1 group M(different subtypes), HIV-1group N, HIV-1group O and HIV-2.

The GB HIV Ag-Ab COMB kit can detect HIV- p24 from different genotypes from type 1 including M,N and O and from type 2.

6.3 Evalution of Reproducibility

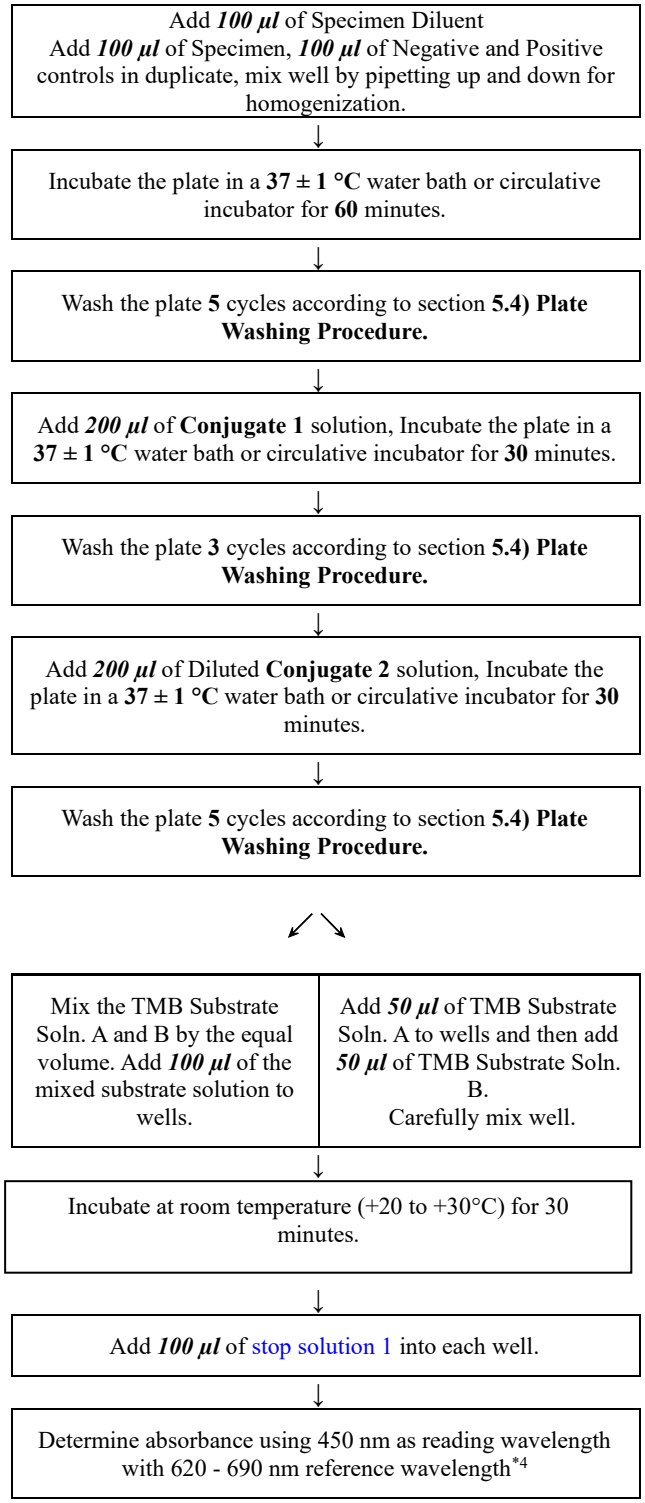
6.3.1 Single-Site Study

Item	Mean	SD	CV%	Specification	Inspection
NC	0.072	0.016	22.38%	≤ 30%	OK
PC1	3.623	0.221	6.09%	≤ 15%	OK
PC2	3.865	0.134	3.46%	≤ 15%	OK
PC3	3.516	0.256	7.29%	≤ 15%	OK
Ab-1 high	2.711	0.245	9.03%	≤ 15%	OK
Ab-1 low	0.515	0.059	11.44%	≤ 20%	OK
Ab-2 high	3.365	0.307	9.13%	≤ 15%	OK
Ab-2 low	0.643	0.128	19.86%	≤ 20%	OK
Ag high	2.969	0.307	10.34%	≤ 15%	OK
Ag low	0.569	0.107	18.88%	≤ 20%	OK

6.3.2 Multiple-Site Study

Item	Mean	SD	CV%	Specification	Inspection
NC	0.067	0.018	26.67%	≤ 30%	OK
PC1	3.725	0.153	4.10%	≤ 15%	OK
PC2	3.775	0.175	4.64%	≤ 15%	OK
PC3	3.478	0.212	6.11%	≤ 15%	OK
Ab-1 high	2.756	0.241	8.75%	≤ 15%	OK
Ab-1 low	0.542	0.061	11.21%	≤ 20%	OK
Ab-2 high	3.387	0.289	8.54%	≤ 15%	OK
Ab-2 low	0.589	0.100	17.00%	≤ 20%	OK
Ag high	2.930	0.317	10.83%	≤ 15%	OK
Ag low	0.568	0.105	18.50%	≤ 20%	OK

7) Flow chart of the test procedure



8) Bibliography

8.1). John W. Gnann, 1987. Synthetic Peptide Immunoassay Distinguishes HIV Type I and HIV Type 2 Infections, Science, 237, 346-349.

8.2). The reference wavelength of spectrometer could be 620nm to 690nm. However, user should validate the spectrometer in combination with this kit before use.

8.3). Gold, J. and Dwyer, J., 1994. A Short History of AIDS. Med. J. Aust. 160:251-252.

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8.5). Saville, R.D., Constantine, N.T., Cleghorn, F.R., Jack, N., Bartholomew, C., Edwards, J., Gomez, P. and Blattner, W.A. 2001. Fourth-generation enzyme-linked immunosorbent assay for the simultaneous detection of human immunodeficiency virus antigen and antibody. J. Clin. Microbiol. 39 (7): 2518-2524.

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8.8). IVD Directive 98/79/CE, Common Technical Specifications (CTS) – Annex II, List A.

SYMBOLS USED ON LABELS			
	Manufacturer		Consult Instructions for Use
IVD	In Vitro Diagnostic Medical Device		Caution
LOT	Batch code		Danger
	Use By		Danger
	Temperature limitation		Warning
CE	CE Mark		Biohazard
REF	Catalogue number		Keep away from Sunlight
EC REP	Authorized representative		

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Date: 07th March 2024.

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We hereby certify that,

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Republic of Moldova,
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Fax:-00-373-22 62 30 32
E-mail: sanmedico.office@gmail.com

have been appointed by us as our **Authorized Distributor** for selling our Products in **MOLDOVA**

This certificate is valid upto 06th March 2026.

This Authorization Letter shall stand effective from the date of signing and can be terminated by either party with two months advance notice.

For HIMEDIA LABORATORIES PVT. LTD.,

HiMedia Laboratories Pvt. Limited Thane 400604

V.M.WARKE.
DIRECTOR – SALES & MARKETING

DECLARATION OF CONFORMITY
MOLECULAR BIOLOGY PRODUCTS

1) Manufacturer *(Name, department)*: **HiMedia Laboratories Pvt. Ltd.**

ADDRESS: PLOT NO. C-40, ROAD NO. 21Y, MIDC, WAGLE INDUSTRIAL AREA, THANE,(WEST) 400604, MAHARASHTRA, INDIA.

and

2) European authorized representative: **CEpartner4U BV,**

Address: **ESDOORNLAAN 13, 3951DB MAARN, THE NETHERLANDS**;
(on product labels printed as:
CEpartner4U , ESDOORNLAAN 13, 3951DB MAARN, THE NETHERLANDS. www.cepartner4u.eu)

3) Product(s) *(groupnames /.)*:

Group	Group name	NL registration no.	No.
MBP	Molecular Biology Products Subgroups: DNA & RNA Isolation Kits, Latex Agglutination Kits, Haematology Kits, PCR Kits	NL-CA002-2013-26447	7

type and model numbers: see appendix

4) The product(s) described above is in conformity with:

Title	Document No.
In vitro Diagnostic Medical Devices Directive	98/79/EC

5) Additional information *(Conformity procedure, Notified Body, CE certificate, Registration nr., etc.)*:
Conformity assessment procedure for CE marking: *In vitro* Diagnostic Medical Device Directive, Annex III

Mumbai, India; 14/04/2022

(Place & date of issue (yyyy-mm-dd))

Dr. G.M.Warke , Managing Director
(name; function and signature of manufacturer)

Appendix

Date: 14/04/2022

List of devices:

Product group	Type/ Model / Ref number	Device Name	Risk Class	Date of CE compliance
Molecular Biology Products				
MBP- DNA Isolation Kits	MB541	HiPurA™ SPP Blood DNA Isolation Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB504	HiPurA™ Blood Genomic DNA Miniprep Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB506	HiPurA™ Mammalian Genomic DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB516	HiPurA™ Blood Genomic DNA Midiprep Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB517	HiPurA™ Blood Genomic DNA Maxiprep Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB521	HiPurA™ 96 Blood Genomic DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB522	HiPurA™ Sperm Genomic DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB525	HiPurA™ Bone DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB531	HiPurA™ Buccal DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB544	HiPurA™ Stool DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB545	HiPurA™ Mycobacterium tuberculosis DNA Purification Kit	Low risk	20/12/2012
MBP- DNA Isolation Kits	MB545D	HiPurA™ Mycobacterium tuberculosis Decontamination Kit	Low risk	28/04/2017
MBP- DNA Isolation Kits	MB554	HiPurA™ Multi-Sample DNA Purification Kit	Low risk	28/04/2017
MBP-RNA Extraction Kits	MB615	HiPurA™ Viral RNA Purification Kit	Low risk	28/04/2017
MBP-RNA Extraction Kits	MB617	HiPurA™ All Blood RNA Purification Kit	Low risk	25/08/2016
MBP- DNA Isolation Kits	MB573	HiPurA™Urine DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MB575	HiPurA™ Viral DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MB578	HiPurA™ QuickBone DNA Purification Kit (Column Based)	Low risk	22/04/2019
MBP- DNA Isolation Kits	MB579	HiPurA™ Fast MTB (Mycobacterium tuberculosis) Genomic DNA Purification Kit	Low risk	22/04/2019
MBP- Latex Agglutination Kits	LK01	HiClostridium™ difficile Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK02	HiSalmonella™ Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK03	HiStaph™ Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK04	HiLegionella™ Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK06	HiStrep™ Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK07	HiListeria™ Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK08	HiRotavirus™ Latex Test Kit	Low risk	20/12/2012
MBP- Latex Agglutination Kits	LK13	HiE.coli™ Latex Test Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR001	Campylobacter Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR002	E. coli O157:H7 Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR003	Vibrio cholerae Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR004	Salmonella Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR005	Listeria Detection Kit	Low risk	20/12/2012

MBP- PCR Kits	MBPCR006	Legionella Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR008	Methicillin Resistant Staphylococcus aureus (MRSA) Detection Kit (Uniplex)	Low risk	20/12/2012
MBP- PCR Kits	MBPCR009	Mycobacterium Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR014	Beta-Thalassemia Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR014A	Beta Thalassemia Detection Kit (Uniplex)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR016	Malaria Detection Kit (Multiplex)	Low risk	20/12/2012
MBP- PCR Kits	MBPCR017	Mycobacterium Detection Kit	Low risk	20/12/2012
MBP- PCR Kits	MBPCR019	Staphylococcus Casette Chromosome (SCC) mec typing MRSA Detection Kit (Multiplex)	Low risk	12/08/2015
MBP- PCR Kits	MBPCR020	Methicillin Resistant Staphylococcus aureus (MRSA) Detection Kit (Multiplex)	Low risk	12/08/2015
MBP- PCR Kits	MBPCR022	Multi-Drug Resistant Mycobacterium Tuberculosis Detection Kit (Multiplex)	Low risk	12/08/2015
MBP- PCR Kits	MBPCR023	E. coli O157:H7 Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR024	Salmonella Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR025	Listeria monocytogenes Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR026	Campylobacter jejuni Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR027	Vibrio cholerae Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR028	Legionella pneumophila Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR029	Chronobacter sakazakii Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR030	Chronobacter sakazakii Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR032	Palm E.coli O157:H7 Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR033	Palm Salmonella Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR034	Palm Mycobacterium tuberculosis Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR035	Palm Malaria Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR036	Palm Listeria monocytogens Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR037	Palm Multi-Drug Resistant Mycobacterium Tuberculosis (MDR-TB) Detection Kit	Low risk	12/08/2015
MBP- PCR Kits	MBPCR038	Acinetobacter baumanni Detection kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR039	Diarrhoegenic E.coli (Multiplex) Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR040	Palm Bacillus subtilis Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR041	Palm Candida albicans Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR042	Palm Pseudomonas aeruginosa Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR043	Palm Staphylococcus aureus Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR044	Klebsiella pneumoniae Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR045	Palm Generic E.coli Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR046	Palm Diarrhoegenic E.coli Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR047	Palm Shigella Spp. Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR049	Palm Klebsiella pneumoniae Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR050	Pseudomonas aeruginosa Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR051	Generic E.coli Detection Kit	Low risk	25/08/2016

MBP- PCR Kits	MBPCR052	Candida albicans Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR053	Bacillus subtilis Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR054	Shigella spp. Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR056	Staphylococcus aureus Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR057	Klebsiella pneumoniae Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR058	Pseudomonas aeuroginosa Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR059	Generic E.coli Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR060	Candida albicans Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR061	Bacillus subtilis Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR062	Shigella Spp. Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR064	Staphylococcus aureus Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR065	Acinetobacter baumanni Detection kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR066	Palm Acinetobacter baumanni Detection kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR067	Palm Clostridium sporogenes Detection kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR068	Palm Salmonella enterica subsp. enterica Detection kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR069	Palm Aspergillus brasilensis Detection kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR073	Multi-Drug Resistant Mycobacterium tuberculosis Detection Kit (Uniplex)	Low risk	28/04/2017
MBP- PCR Kits	MBPCR074	Clostridium sporogenes Detection Kit	Low risk	28/04/2017
MBP- PCR Kits	MBPCR075	Zika Detection Kit (One Step Real time PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR076	Zika Detection Kit (One Step Semi quantitative PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR077	Zika Detection Kit (Two Step Semi quantitative PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR078	Palm Zika Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR079	Dengue Detection Kit (One Step Real time PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR080	Dengue Detection Kit (One Step Semi quantitative PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR081	Dengue Detection Kit (Two Step Semi quantitative PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR082	Palm Dengue Detection Kit (One Step RT-PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR083	Chikungunya Detection Kit (One Step Real time PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR084	Chikungunya Detection Kit (One Step Semi quantitative PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR085	Chikungunya Detection Kit (Two Step Semi quantitative PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR086	Palm Chikungunya Detection Kit	Low risk	25/08/2016
MBP- PCR Kits	MBPCR092	Malaria Detection Kit (Real-Time PCR)	Low risk	25/08/2016
MBP- PCR Kits	MBPCR099	Salmonella Detection Kit (Real-Time Probe Based PCR)	Low risk	28/04/2017
MBP- PCR Kits	MBPCR101	Generic Dengue Detection Kit (Real-Time Probe Based PCR)	Low risk	28/04/2017
MBP- PCR Kits	MBPCR102	Generic Dengue Detection Kit (One-Step Semi-Q PCR Based)	Low risk	28/04/2017
MBP- PCR Kits	MBPCR103	Generic Dengue Detection Kit (One-Step Real-Time SYBr based PCR)	Low risk	28/04/2017
MBP- PCR Kits	MBPCR104	Leptospira Detection Kit (Real Time PCR Probe Based)	Low risk	28/04/2017
MBP- PCR Kits	MBPCR108	Mycobacterium tubercuosis Detection Kit (Real Time Probe Based PCR)	Low risk	16/12/2017

MBP- PCR Kits	MBPCR111	Malaria Detection Kit (Real Time Probe Based PCR)	Low risk	16/12/2017
MBP- PCR Kits	MBPCR112	Chikungunya Detection Kit (Real Time Probe Based PCR)	Low risk	16/12/2017
MBP- PCR Kits	MBPCR130	Palm MRSA Detection Kit	Low risk	16/12/2017
MBP- PCR Kits	MBPCR131	Extended Spectrum β-Lactamase Typing Kit (Multiplex) (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR132	Carbapenemase Resistance Typing Kit (Multiplex) (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR133	Methicillin Resistant Staphylococcus aureus Detection Kit (Multiplex) (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR134	Vancomycin Resistant Enterococci (VRE) Detection Kit (Multiplex)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR135	Plasmodium Species Detection Kit (Multiplex) (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR140	Legionella species Detection Kit (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR151	Rotavirus Detection Kit (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR152	Campylobacter species Detection Kit (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR157	Acute Febrile Illness Panel Detection Kit (Real-Time Probe Based PCR)	Low risk	04/07/2018
MBP- PCR Kits	MBPCR159	Sepsis Pathogen Detection Kit (Multiplex) (Semi-Q PCR Based PCR Kit)	Low risk	22/04/2019
MBP- PCR Kits	MBPCR180	Salmonella enterica subsp. enterica Detection Kit	Low risk	22/04/2019
MBP- Haematology Kits	MBP001	Alkaline Hemoglobin Electrophoresis Kit	Low risk	20/12/2012
MBP- Haematology Kits	MBP003	Hi-Speed Sickle Kit (Centrifugation based detection of Hemoglobin 'S')	Low risk	20/12/2012
MBP- Haematology Kits	MBP006	Hi-Super Speed Sickle Kit (Solubility Test for detection of Hemoglobin 'S')	Low risk	20/12/2012
MBP- Haematology Kits	MBP007	Hi- Sickle Kit(Solubility Test)	Low risk	28/04/2017
MBP- Haematology Kits	MBP008	Alkaline Haemoglobin Electrophoresis Kit	Low risk	25/08/2016
MBP- Haematology Kits	MBP009	Alkaline Paper Hemoglobin Electrophoresis Kit	Low risk	16/12/2017
MBP- Haematology Kits	MBP010	Hi-Thal Kit	Low risk	16/12/2017
MBP- DNA Isolation Kits	MBIN001	InstaNX™ Blood Genomic DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MBIN003	Insta NX® Tissue Genomic DNA Purification Kit	Low risk	22/04/2019
MBP- DNA Isolation Kits	MBIN004	InstaNX™ Urine DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MBIN007	InstaNX™ Stool DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MBIN011	Insta NX® Cell Genomic DNA Purification Kit	Low risk	22/04/2019
MBP- DNA Isolation Kits	MBIN012	InstaNX™ Bone DNA Purification Kit	Low risk	04/07/2018
MBP-RNA Extraction Kits	MBIN013	InstaNX™ Viral RNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MBIN015	InstaNX™ Viral DNA Purification Kit	Low risk	04/07/2018
MBP- DNA Isolation Kits	MBIN017	Insta NX® Mycobacterium tuberculosis DNA Purification Kit	Low risk	22/04/2019
MBP- DNA Isolation Kits	MBIN019	Insta NX® Mycobacterium tuberculosis DNA Purification Kit	Low risk	22/04/2019
MBP- DNA Isolation Kits	MBIN021	Insta NX® Blood RNA Purification Kit	Low risk	22/04/2019
MBP - RNA Isolation Kits	MB618	HiPurA® Tissue RNA purification kit	Low risk	09/09/2020
MBP - DNA Isolation Kits	MB582	HiPurA® Viral DNA/RNA purification kit	Low risk	09/09/2020

MBP - DNA Isolation Kits	MB583	HiPurA® DNA/RNA purification kit	Low risk	09/09/2020
MBP - RNA Isolation Kits	MB615M	HiPurA® viral RNA Purification kit (Magnetic Bead Based)	Low risk	09/09/2020
MBP - DNA Isolation Kits	MBIN025	Insta NX® Blood DNA plus purification kit	Low risk	09/09/2020
MBP - DNA Isolation Kits	MBIN026	Insta NX® Viral DNA plus purification kit	Low risk	09/09/2020
MBP - RNA Isolation Kits	MBIN027	Insta NX® Tissue/cell RNA purification kit	Low risk	09/09/2020
MBP- Haematology Kits	MBP009	Alkaline paper haemoglobin electrophoresis kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR137	Hi-PCR® Dengue Serotyping Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR238	Hi-PCR® Generic E. coli Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR154	Hi-PCR® E.coli O157:H7 Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR156	Hi-PCR® Vibrio cholerae Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR201	Hi-PCR® Enterovirus Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR196	Hi-PCR® Salmonella Quantification Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR198	Hi-PCR® E. coli O157:H7 Quantification Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR209	Hi-PCR® Colistin Resistance Probe PCR Kit	Low risk	09/09/2020
MBP - RNA Isolation Kits	MB615MA	HiPurA® viral RNA Automated RNA extraction kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR153	Hi-PCR® Generic E.coli Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR200	Hi-PCR® Total Coliform Probe PCR Kit	Low risk	09/09/2020
MBP - PCR Kits	MBPCR243	Hi-PCR® Coronavirus (COVID-19) Multiplex Probe PCR Kit	Low risk	09/09/2020
MBP - RNA Isolation Kits	MB615NB32200	HiPurA® Pre-Filled plates for Insta NX® Mag32 (For 200ul Sample Volume)	Low risk	01/10/2020
MBP - RNA Isolation Kits	MB615MPF-32	HiPurA® Pre-Filled plates for Insta NX® Mag32	Low risk	19/12/2020
MBP - RNA Isolation Kits	MB615MPF-96	HiPurA® Pre-Filled plates for Insta NX® Mag96	Low risk	19/12/2020
MBP - RNA Isolation Kits	MB615MPF-T	HiPurA® Pre-Filled Medium plates-T	Low risk	19/12/2020
MBP - RNA Isolation Kits	MB615MPF96-200	HiPurA® Pre-Filled plates for Insta NX® Mag96 (For 200ul Sample Volume)	Low risk	19/12/2020
MBP - RNA Isolation Kits	MB615MPF32-200	HiPurA® Pre-Filled plates for Insta NX® Mag32 (For 200ul Sample Volume)	Low risk	19/12/2020
MBP - RNA Isolation Kits	MB615MPF-G	P HiPurA® re-Filled Medium plates-G	Low risk	18/09/2020
MBP - RNA Isolation Kits	MB615MPF-T1	HiPurA® Pre-Filled Medium plates-T	Low risk	24/09/2020
MBP - RNA Isolation Kits	MB615MPF-T4	HiPurA® Pre-Filled Medium plates-T	Low risk	24/09/2020
MBP - RNA Isolation Kits	MB615MPF3211	HiPurA® Super 11 Pre-Filled plates for Insta NX® Mag32	Low risk	05/11/2020
MBP - RNA Isolation Kits	MB615MPF9611	HiPurA® Super 11 Pre-Filled plates for Insta NX® Mag96	Low risk	05/11/2020
MBP - RNA Isolation Kits	MB615MAC-96	HiPurA® Viral RNA Automated extraction combi kit	Low risk	01/10/2020
MBP - RNA Isolation Kits	MB615MAC-32	HiPurA® Viral RNA Automated extraction combi kit	Low risk	01/10/2020
MBP - PCR Kits	MBPCR243A	HiPCR® Coronavirus (COVID-19) Multiplex Probe PCR kit 2.0	Low risk	15/02/2021
MBP - RNA Isolation Kits	MB615MPF-Z	HiPurA® Pre- filled Medium Plates – Z	Low risk	17/03/2021
MBP - RNA Isolation Kits	MB615MPF-48	HiPurA® Pre- filled Plates for Insta NX® Mag96	Low risk	17/03/2021

MBP - RNA Isolation Kits	MB615MPF-96H	HiPurA® Viral RNA Purification Kit (For Insta NX® Mag 96)	Low risk	17/03/2021
MBP - RNA Isolation Kits	MB554MPF96-200	HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag96	Low risk	18/03/2021
MBP - RNA Isolation Kits	MB554MPF32-200	HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32	Low risk	18/03/2021
MBP	LA1092-1NO	Salivol™- Container for Sputum & Saliva Sample	Low risk	18/03/2021
MBP	LA1095-1NO	Stoocol™ - Container for Stool Sample	Low risk	18/03/2021
MBP - RNA Isolation Kits	MB615MPF9611-C	HiPurA® Super 11 Pre- filled Plates for Insta NX® Mag96 (with carred RNA)	Low risk	07/04/2021
MBP - RNA Isolation Kits	MB615MPF-A	HiPurA® Pre- filled Medium Plates -A	Low risk	07/04/2021
MBP - RNA Isolation Kits	MB615MPF-B	HiPurA® Pre- filled Medium Plates -B	Low risk	07/04/2021
MBP - RNA Isolation Kits	MB615MF-32-10NO	Pre- filled HiPurA® Viral RNA Purification Kit (Magnetic Bead Based)	Low risk	14/06/2021
MBP - RNA Isolation Kits	MB615MF-96-5NO	Pre- filled HiPurA® Viral RNA Purification Kit (Magnetic Bead Based)	Low risk	14/06/2021
MBP - RNA Isolation Kits	MB615MFG-96-5NO	Pre- filled HiPurA® Viral RNA Purification Kit (Magnetic Bead Based)	Low risk	14/06/2021
MBP - RNA Isolation Kits	MB615MFT-96-5NO	Pre- filled HiPurA® Viral RNA Purification Kit (Magnetic Bead Based)	Low risk	14/06/2021
MBP - RNA Isolation Kits	MB615MPFNB32	HiPurA® Pre- filled Plates for Insta NX® Mag32 (For upto 400µl sample volume)	Low risk	14/06/2021
MBP - RNA Isolation Kits	MB615MPF9611-H	HiPurA® Super 11 Magnetic Pre-filled Kit	Low risk	14/06/2021
MBP - RNA Isolation Kits	MB615MAC-96T	HiPurA® Viral RNA Automated Extraction Combi Kit	Low risk	14/06/2021
MBP- PCR Kits	MBPCR255	Hi-PCR® COVID-19 Triplex Probe PCR Kit	Low risk	08/12/2021
MBP- PCR Kits	MBT189	Hi-PCR® Covid-19 Detection Kit (Dry Swab Method)	Low risk	08/12/2021
MBP- PCR Kits	MBPCR260	Hi-PCR® RAPID SARS-CoV-2 Multiplex Probe PCR Kit	Low risk	21/12/2021
MBP - RNA Isolation Kits	MB615MPFZ1	HiPurA® Pre- filled Medium Plates –Z1	Low risk	21/12/2021
MBP - RNA Isolation Kits	MB615MPF-16D	HiPurA® Pre- filled Plates for Viral RNA Extraction - 16	Low risk	15/02/2022
MBP - RNA Isolation Kits	MB615PC1611	HiPurA® Pre- filled Cartridges for Viral RNA Extraction	Low risk	15/02/2022

THE INTERNATIONAL CERTIFICATION NETWORK

CERTIFICATE

Quality Austria
has issued an IQNet recognized certificate that the organization:

HiMedia Laboratories Pvt. Ltd.
Plot NO. C40, ROAD - 21Y, WAGLE INDUSTRIAL ESTATE, THANE (WEST) - 400604 MAHARASHTRA, INDIA

for the following scope:

Design, Development & Testing of Microbiology, Animal Cell Culture, Plant Tissue Culture & Molecular Biology products

EAC: 34

has implemented and maintains a

QUALITY MANAGEMENT SYSTEM

which fulfils the requirements of the following standard

ISO 9001:2015

This attestation is directly linked to the IQNet Partner's original certificate and shall not be used as a stand-alone document

Issued on:	2022-02-28
Validity date:	2025-02-27
Quality Austria certified since:	2022-02-28

Registration Number: AT-27302/0

Alex Stoichitoiu
President of IQNet

Mag. Friedrich Khuen-Belasi
Authorised Representative of Quality Austria

PARTNER OF IQNet

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Quality Austria - Trainings, Zertifizierungs und Begutachtungs GmbH awards this **quality**austria certificate to the following organisation:

This **quality**austria certificate confirms the application and further development of an effective

HiMedia Laboratories Pvt. Ltd.
Plot NO. C40, Road - 21Y, Wagle Industrial Estate,
Thane (West) - 400604 Maharashtra, INDIA

QUALITY MANAGEMENT SYSTEM
complying with the requirements of standard
ISO 9001:2015

Design, Development & Testing of Microbiology, Animal Cell Culture, Plant Tissue Culture & Molecular Biology products

Registration No.: 27302/0
Date of initial issue: 28 February 2022
Valid until: 27 February 2025

The validity of the **quality**austria certificate will be maintained by annual surveillance audits and one renewal audit after three years.

Vienna, 28 February 2022

Quality Austria - Trainings, Zertifizierungs und Begutachtungs GmbH,
AT-1010 Vienna, Zelinkagasse 10/3

Mag. Christoph Mondl
General Manager

Mag. Dr. Werner Paar
General Manager

Mag. Dr. Anni Koubek
Specialist representative

Dok. Nr. FO_24_028

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The current validity of the certificate is documented exclusively on the Internet under
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THE INTERNATIONAL CERTIFICATION NETWORK

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Quality Austria
has issued an IQNet recognized certificate that the organization:

HiMedia Laboratories Pvt. Ltd.
Plot NO. C40, ROAD - 21Y, WAGLE INDUSTRIAL ESTATE, THANE (WEST) - 400604 MAHARASHTRA, INDIA

for the following scope:

Design, Development & Testing of Biosciences Products for application in Microbiology, Animal Cell Culture & Molecular Biology products

EAC: 34

has implemented and maintains a

QUALITY MANAGEMENT SYSTEM

which fulfils the requirements of the following standard

ISO 13485:2016

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Issued on:	2022-02-28
Validity date:	2025-02-27
Quality Austria certified since:	2022-02-28

Registration Number: AT-00391/0

PARTNER OF IQNet

Alex Stoichitoiu
President of IQNet

Mag. Friedrich Khuen-Belasi
Authorised Representative of Quality Austria

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Quality Austria - Trainings, Zertifizierungs und Begutachtungs GmbH awards this **quality**austria certificate to the following organisation:

This **quality**austria certificate confirms the application and further development of an effective

QUALITY MANAGEMENT SYSTEM
complying with the requirements of standard
ISO 13485:2016
Medical devices - Quality management systems - Requirements for regulatory purposes

HIMEDIA

HiMedia Laboratories Pvt. Ltd.

Plot NO. C40, Road - 21Y, Wagle Industrial Estate,
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Design, Development & Testing of Biosciences Products for application in Microbiology, Animal Cell Culture & Molecular Biology products

Registration No.: 00391/0

Date of initial issue: 28 February 2022

Valid until: 27 February 2025

The validity of the **quality**austria certificate will be maintained by annual surveillance audits and one renewal audit after three years.

Vienna, 28 February 2022

Quality Austria - Trainings, Zertifizierungs und Begutachtungs GmbH,
AT-1010 Vienna, Zelinkagasse 10/3

Mag. Christoph Mondl	Mag. Dr. Werner Paar	Mag. Dr. Anni Koubek
General Manager	General Manager	Specialist representative

Dok. Nr. FO_24_028

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The current validity of the certificate is documented exclusively on the Internet under
http://www.qualityaustria.com/en/cert EAC: 34

MB615 HiPurA® Viral RNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB615		
		20 Preps	50 preps	250 preps
DS0037	RNA Lysis Solution (HRL)	16 ml	40 ml	200 ml
DS0012	Wash Solution Concentrate (WS)	6 ml	15 ml	75 ml
DS0042	Elution Solution (RNase- Free Water)	2.4 ml	6 ml	30 ml
DS0192	Carrier RNA	0.28 mg	0.7 mg	3.5 mg
DBCA03	HiElute Miniprep Spin Column (Capped) [in Uncapped Collection Tube]	20 nos	50 nos	250 nos
PW146	Micro Centrifugal Tube-B (1.5ml)	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	20 nos	50 nos	250 nos

Intended Use

Recommended for isolation of Viral RNA from various samples like fresh and frozen plasma, serum, nasopharyngeal swab, oropharyngeal swab, sputum, BAL in Viral Transport Medium and other body fluids.

Introduction

HiPurA® Viral RNA Purification Kit provide the fastest and easiest way to purify viral RNA for reliable use in amplification technologies. Viral RNA can be purified from plasma (treated with anticoagulant EDTA), serum, other body fluids, and infected tissues. Samples may be fresh or frozen, but if frozen, should not be thawed more than once. Repeated freeze–thawing of plasma samples will lead to reduced viral titers and should be avoided for optimal sensitivity. HiPurA® Viral RNA Purification Kit can be used for isolation of viral RNA from a wide variety of viruses, but performance may vary depending on virus type.

ISO 9001-2015 CERTIFIED WHO GMP CERTIFIED ISO 13485-2016 CERTIFIED CE

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HiPurA® Viral RNA Purification Kit

This kit carries out efficient extraction of viral RNA from wide range of viral strains like Influenza**,** Dengue, Chikungunya and viral pathogen of animals. Sample is first lysed under the highly denaturing conditions provided by Buffer HRL to inactivate RNases and to ensure isolation of intact viral RNA. When Carrier RNA is added to Elution Solution (RNase-free Water), it improves the binding of viral RNA to the HiElute Miniprep Spin Column especially in the case of low-titer samples, and limits possible degradation of the viral RNA due to any residual RNase activity.

Elution

The yield of RNA depends on the sample type and the number of cells in the sample. A single elution with 60-80µl of Elution Solution will provide sufficient RNA to carry out multiple amplification reactions.
NOTE: For more concentrated RNA lower elution volume (30-40 µl) can be used. Larger elution volumes (up to 100 µl) can also be used but may result in dilution of viral RNA sample.

HiElute Miniprep Spin Column (Capped) (DBCA03)

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced gel membrane and the speed plus versatility of spin column technology to yield high quantity of RNA. The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins, and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional RNA isolation techniques. RNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit.

Storage

HiPurA® Viral RNA Purification Kit can be stored at room temperature (15-25°C) for up to 1 year without showing any reduction in performance. Store the DS0192- Carrier RNA in -20°C temperature on receipt. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

Materials needed but not provided

- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- RNase – free pipette tips (aerosol barrier recommended)
- Ethanol (96 – 100%)

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non- disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.
2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.
3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.
4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240°C for four or more hours before use. Alternatively, glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.
5. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
6. Solutions (water and other solutions) should be treated with 0.1% DEPC

General Preparation Instructions

1. **Thoroughly mix reagents**
   Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

2. Ensure that clean & dry Nuclease-free tubes and tips are used for the procedure.

3. **Reconstitute Carrier RNA**

Number of Preps	Carrier RNA	Elution Buffer (RNase free water)
20	0.28 mg	280 µl
50	0.7 mg	700 µl
250	3.5 mg	3.5 ml

Dissolve Carrier RNA thoroughly by pipetting. We recommend storing the reconstituted Carrier RNA at -20°C in aliquots to avoid repeated freeze and thaw.

4. **Preparation of Carrier RNA –Lysis Solution (HRL)**

Number of Preps	Volume of Carrier RNA	Volume of Lysis Solution (HRL)
20	112 µl	11.2 ml
50	280 µl	28 ml
250	1.4 ml	140 ml

NOTE: Concentration of Carrier RNA to be used is 10µg/ml

Calculate the volume of Carrier RNA –Lysis Solution (HRL) as follows:

a X 0.56 ml = **b** ml

b ml X 10 µl/ml = **c** µl

where, **a** = number of sample to be processed

b = volume of Lysis Solution (HRL) to be added for '**a**' number of samples

c = volume of Carrier RNA to be added to Lysis Buffer (HRL)

eg: for 2 number of samples, add 1.12 ml of Lysis Solution (HRL) and 11.2 µl of Carrier RNA

Number of Preps	Lysis Solution (HRL) ml	Reconstituted Carrier RNA µl	Number of Preps	Lysis Solution (HRL) ml	Reconstituted Carrier RNA µl
1	0.56	5.6	13	7.28	72.8
2	1.12	11.2	14	7.84	78.4
3	1.68	16.8	15	8.40	84.0
4	2.24	22.4	16	8.96	89.6
5	2.80	28.0	17	9.52	95.2
6	3.36	33.6	18	10.08	100.8
7	3.92	39.2	19	10.64	106.4
8	4.48	44.8	20	11.20	112.0
9	5.04	50.4	21	11.76	117.6
10	5.60	56	22	12.32	123.2
11	6.16	61.6	23	12.88	128.8
12	6.72	67.2	24	13.44	134.4

5. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	6 ml	18 ml
50	15 ml	45 ml
250	75 ml	225 ml

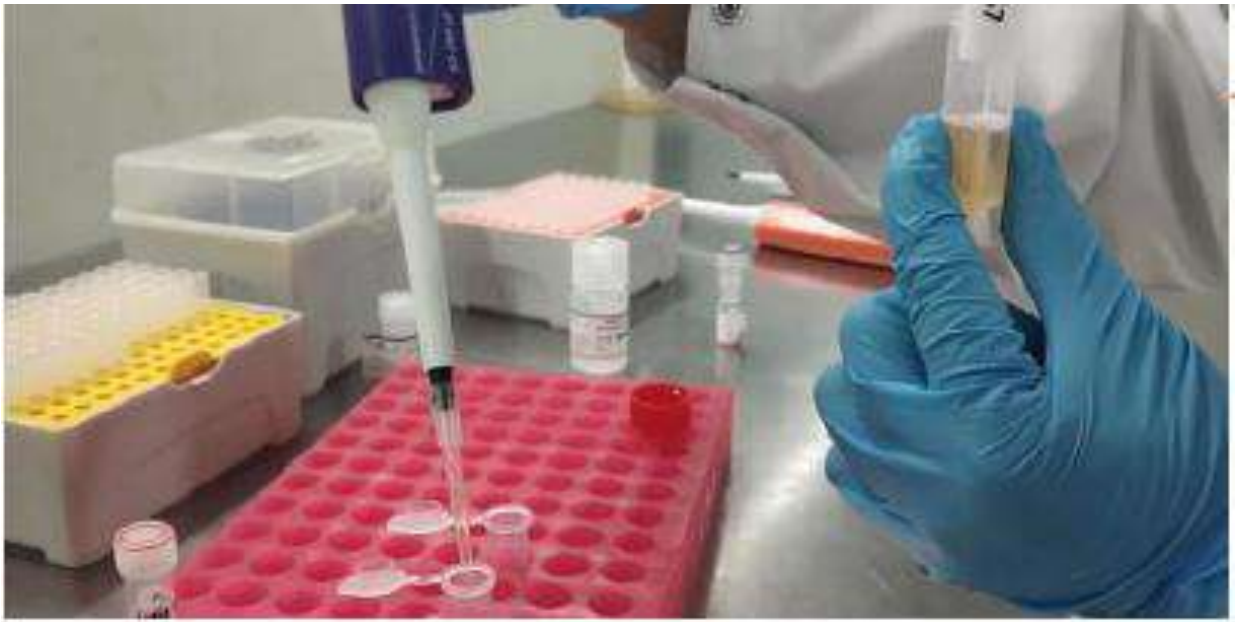
Specimen Handling and Collection

Collect plasma, serum or other body fluids in a sterile container. Thaw the samples on ice before use.
Repeated freeze- thaw of samples should be avoided.

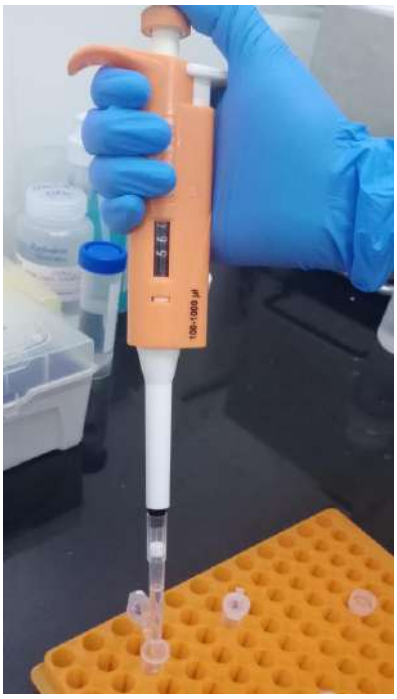
Type of Specimens: Clinical samples (Serum, plasma, swabs in viral transport medium and other body fluids)

Procedure

1. Add 140 µl of sample like serum, plasma or body fluid, nasopharyngeal swab, oropharyngeal swab, sputum, BAL, samples collected in Viral Transport medium to Collection Tube, Polypropylene (2.0 ml) (PW1139).
   NOTE: The procedure is optimized for use with 140 µl samples but upto 300 µl sample can be used.
   During work with smaller sample volume (~100 µl) it should be made up to 140 µl with PBS (Phosphate Buffered Saline) before processing. If the initial sample volume is increased the amount of RNA Lysis Solution should be increased proportionally and application of the lysed sample to the HiElute Miniprep Spin Column will require multiple loading steps.



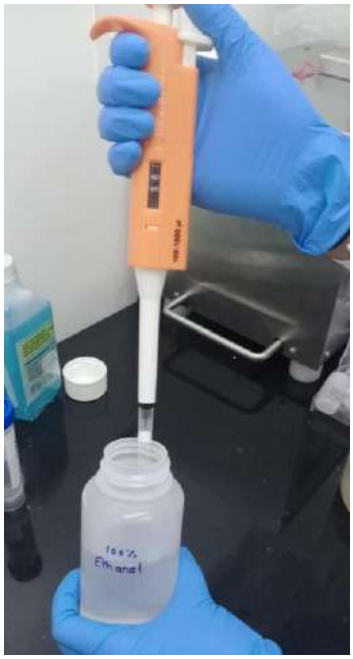
2. Add 560 µl of Carrier RNA-Lysis Solution (HRL) to the sample. (**Refer to General Preparation Instructions)**. Mix by pulse vortexing for 15 seconds.



3. Incubate for 10 minutes at room temperature (15-25°C).

4. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.

5. **Binding**
   Add 560 µl of ethanol (96-100%) to the sample, mix well by gentle pipetting.

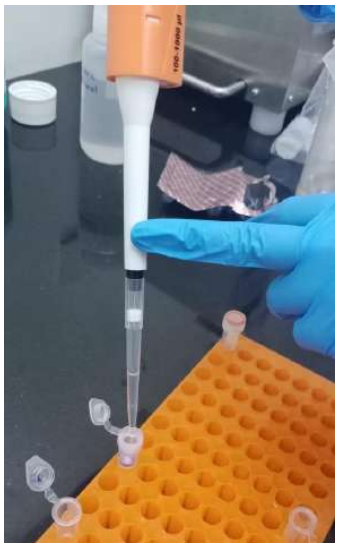



6. Centrifuge the samples for 10 seconds to remove any droplets formed inside the cap of collection tubes.

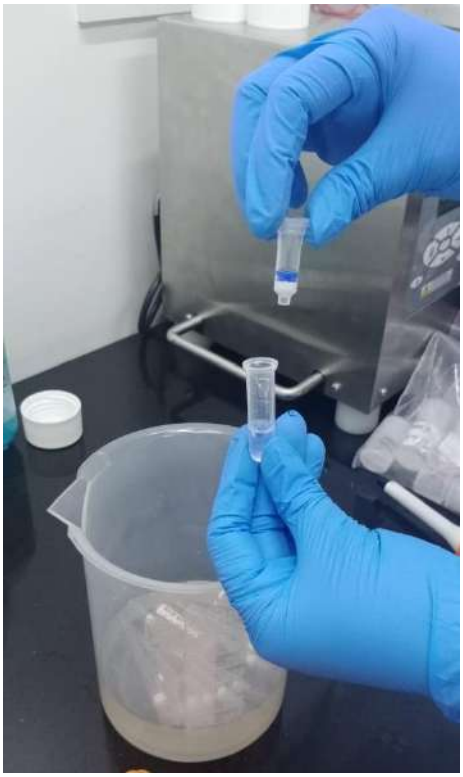
7. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
Transfer the lysate obtained in step 6 onto the HiElute Miniprep Spin Column. Centrifuge at 8,000 rpm for 1 minute.

8. Discard the flow-through after the spin. Repeat step 7 with the remaining sample. Reuse the collection tube.

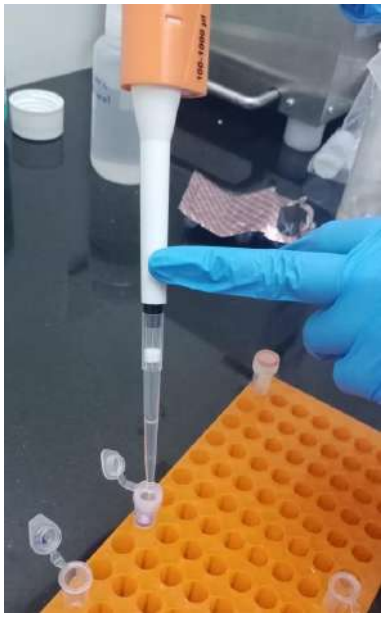
9. **First Wash**
(Prepare Wash Solution as indicated in General Preparation Instructions)
Add 500 µl of diluted Wash Solution (WS) (DS0012). Centrifuge at 8,000 rpm for 1 minute.


10. Discard the flow-through. Reuse the collection tube.



11. **Second Wash**
Add another 500 µl of diluted Wash Solution (WS) (DS0012) onto the column. Close the tube gently and centrifuge for 3 minutes at 14,000 rpm to wash the column.


12. Discard the flow-through. Reuse the collection tube. Centrifuge for 1 minute at 14,000 rpm to dry the membrane.


13. Transfer the HiElute Miniprep Spin column (Capped) (DBCA03) to a Micro Centrifugal Tube 1.5ml (PW146). Pipet 60-80 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Incubate for 1 minutes at room temperature (15-25°C).

14. Close the tube gently and centrifuge for 1 minute at 8,000 rpm. The eluate in the Micro Centrifugal Tube 1.5ml (PW146) contains pure RNA.
NOTE: Place the Micro Centrifugal Tube 1.5ml (PW146) at alternate position in the rotor of the centrifuge machine. The cap of the Micro Centrifugal Tube 1.5ml (PW146) might break if kept side by side in the rotor of centrifuge machine.

Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

Warning

Certified for *in vitro* Diagnostic Use (IVD). Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Performance and Evaluation

The yield and efficiency of purification is determined by performing Real- Time PCR. Yield of viral RNA isolated from biological samples is usually very less (approx. <1 µg). As a result it is difficult to measure the yield spectrophotometrically. Another point to keep in mind that the carrier RNA will account for most of the RNA present. The yield and efficiency of purification is determined by performing Quantitative RT-PCR. All the QC passed batches have at least 90% recovery of the viral RNA.

Quality Control

Each lot of HiMedia's HiPurA® Viral RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

References

1. Sambrook, J.,*et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989)
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993).

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column (Capped)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		Centrifugation temperature is too low	The centrifugation temperature should be 20 – 25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing lysate to 37°C before transferring it to the column.
2.	Low RNA Yield	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the column for 10 minutes at room temperature with Elution solution (RNase free water) before centrifuging.
		Ethanol carryover	During the second wash with Wash Solution (WS) be sure to centrifuge at ≥8000 x g (≥10, 000 rpm) for 2 minutes to dry the column. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur. To eliminate any chance of possible ethanol, centrifuge the column for another step minute at full speed.
		No DNase treatment	Follow the optional on-column DNase digestion
3.	RNA does not perform well in downstream experiments	Ethanol carryover	During the second Wash using Wash Solution (WS), be sure to dry the HiElute Miniprep Spin Column membrane by centrifugation at ≥8000 x g (≥10,000 rpm) for 2 minutes to dry the membrane. Following the centrifugation, remove the HiElute Miniprep Spin Column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

Safety Information

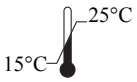
The HiPurA® Viral RNA Purification Kit is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail to mb@himedialabs.com.

IVD	In vitro diagnostic medical device
CE	CE Marking
	Storage temperature
	Do not use if package is damaged
	HiMedia Laboratories Private Limited, Reg. Off: Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Area, Thane, (West) 400604, Maharashtra, INDIA. Web: www.himedialabs.com
EC REP	CE Partner 4U ,Esdoornlaan 13, 3951 DB Maarn The Netherlands, www.cepartner 4u.eu
	08/2025

Please refer disclaimer Overleaf.

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory,diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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Адреса 03179, м. Київ, вул. Академіка Єфремова, 23
Для кореспонденції: 03179, а/с 49
З питань замовлення продукції: 050-422-62-16, 067-422-62-16
Тел.: +38 (095) 60-99-555 Факс: +38 (044) 422-62-16
e-mail: info@xema.com.ua
www.xema.in.ua

STATEMENT

We, XEMA LLC, as a manufacturer of in vitro diagnostic medical devices, having a registered office at Akademika Yefremova St. 23, Kyiv, Ukraine assign SRL SANMEDICO having a registered office at A. Corobceanu Street 7A, apt. 9, Chişinău MD-2012, Moldova, as authorized representative in correspondence with legislative requirements of the Republic of Moldova.

We declare that the company mentioned above is authorized to register, notify, renew, or modify the registration of medical devices on the territory of the Republic of Moldova.

This Statement shall come into force on the date of its signing. The duration of this Statement is 3 years from the date of signing.

Date: 06.09.2023

Signature:

Director Xema LLC
Oleksandra Zavaliei

Polmed.de

Vertretung und Repräsentanz

Certificate

Of Marketing Authorization of Medical Product

within Germany, the member states of the European Union
and the other states having a contractual agreement with the European Economic Area

Nr. AR/IVD/XEMA LLC/01/2023

Issued on the basis of the Declaration of conformity and registration taking into account account Article 11 of Regulation (EU) 2017/746 (IVDR) on In Vitro Diagnostic, and Medical Device Implementing Act (MPDG)

Ausgestellt auf Grund der Konformitätserklärung und Registrierung unter Berücksichtigung der der Verordnung (EU) 2017/746 (IVDR) über In-vitro-Diagnostika und Medizinprodukterecht-Durchführungsgesetz (MPDG)

Manufacturer / Hersteller	**XEMA LLC** **SRN: UA-MF-000032959** UKRAINE, 03179 KYIV Akademika Yefremova St. 23 qa@xema.com.ua; www.xema.in.ua
Product name / Produkt	**See annex to the Certificate** Siehe Anhang zum Zertifikat
Product Classification: Produktklassifizierung	**In Vitro Diagnostic Medical Devices** In-vitro-Diagnostikum (IVD) Medizinprodukte
Category: Kategorie	**Common/ Other IVD** Sonstige IVD-Produkte
Conformity assessment procedure: Konformitatsbewertungsverfahren:	**EC DECLARATION OF CONFORMITY** **(Annex III, except point 6, Directive 98/79/EC)** **in connection with article 110(3) IVDR** **EU- KONFORMITATSERKLARUNG** (Anhang III, außer Nummer 6, Richtlinie 98/79 / EG) in Verbindung mit Artikel 110 (3) IVDR
State Competent Authority: Staatliche Zuständige Behörde	**BfArM** Federal Institute for Drugs and Medical Devices DMIDS (German Medical Device Information and Database System) **BfArM** Das Bundesinstitut für Arzneimittel und Medizinprodukte DMIDS (Deutsches Medizinprodukte-Informations- und Datenbanksystem)

Date of issue : **2023-03-07**
Das Ausstellungsdatum

Valid to : **2025-05-31**
Gültig bis

Represented in the EC by:

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Tel: +49 911 93163967

Beata Rozwadowska * Polmed.de * Fichtenstr. 12a, 90763 Fürth, Germany

Polmed.de

SRN: DE-AR-000006947

Valid with the Extract from the database www.dimdi.de (German Medical Device Information and Database System (DMIDS))
Gilt nur mit :Auszug aus der Datenbank www.dimdi.de (Deutsches Medizinprodukte-Informations- und Datenbanksystem (DMIDS))

Polmed.de

Vertretung und Repräsentanz

Annex to the Certificate No.:

Anhang zum Zertifikat Nr.:

AR/IVD/XEMA LLC/01/2023

The following medical devices can be placed on the market in the Federal Republic of Germany, in the member states of the European Economic Community (EEC) and in the other contract states of the agreement about the European Economic Area.

Die folgenden Medizinprodukte in der Bundesrepublik Deutschland, in den Mitgliedsstaaten der Europäischen Wirtschaftsgemeinschaft (EG) und in den Vertragsstaaten der EG in den Verkehr gebracht werden dürfen.

#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
1.	ASPERGILLUS	K021	GalMAg EIA	**DE/CA64/00115824**
2.	HSV IgG	K104	HSV 1/2 IgG EIA	**DE/CA64/00115826**
3.	HSV IgM	K104M	HSV 1, 2 IgM EIA	**DE/CA64/00115833**
4.	HSV 2 IgG	K104B	HSV 2 IgG EIA	**DE/CA64/00115836**
5.	MYCOPLASMA ANTIBODY ASSAYS	K106	Mycoplasma IgG EIA	**DE/CA64/00115837**
6.	SYPHILIS ANTIBODY ASSAYS TOTAL	K111	anti-Treponema pallidum EIA	**DE/CA64/00115839**
7.	SYPHILIS ANTIBODY IGG	K111G	Treponema pallidum IgG EIA	**DE/CA64/00115840**
8.	H. PYLORI ANTIBODY ASSAYS	K119G	Helicobacter pylori IgG EIA	**DE/CA64/00115850**
9.	OTHER OTHER BACTERIOLOGY IMMUNOASSAY	K126	Ureaplasma IgG EIA	**DE/CA64/00115851**
10.	THYROID PEROXIDASE (INCL. MICROSOMAL) ANTIBODIES	K131	aTPO EIA	**DE/CA64/00115852**
11.	THYROGLOBULIN AUTOANTIBODIES	K132	aTG EIA	**DE/CA64/00115853**
12.	MPO ANCA	K133	aMPO EIA	**DE/CA64/00115854**
13.	TISSUE TRANSGLUTAMINASE ANTIBODIES	K160 K161	anti-TGlu IgG EIA anti-TGlu IgA EIA	**DE/CA64/00115855**
14.	GIARDIA LAMBLIA	K171	anti-Giardia lamblia EIA	**DE/CA64/00115856**
15.	OTHER PARASITOLOGY	K174	Ascaris IgG EIA	**DE/CA64/00115857**
16.	ECHINOCOCCUS	K175	Echinococcus IgG EIA	**DE/CA64/00115858**
17.	DISTOMATOSIS	K176	Opisthorchis IgG EIA	**DE/CA64/00115859**
18.	GLIADIN ANTIBODIES	K180 K181	Gliadin IgG EIA Gliadin IgA EIA	**DE/CA64/00115860**
19.	IMMUNOGLOBULIN E – TOTAL	K200	Total IgE EIA	**DE/CA64/00115861**
20.	THYROID STIMULATING HORMONE	K201	TSH EIA	**DE/CA64/00115863**
21.	LUTEINISING HORMONE	K202	LH EIA	**DE/CA64/00115864**
22.	FOLLICLE STIMULATING HORMONE	K203	FSH EIA	**DE/CA64/00115865**
23.	HUMAN GROWTH HORMONE	K204	GH EIA	**DE/CA64/00115866**
24.	HUMAN CHORIONIC GONADOTROPIN TOTAL	K205	hCG EIA	**DE/CA64/00115867**
25.	PROLACTIN	K206	Prolactin EIA	**DE/CA64/00115868**

The above-mentioned medical products are marked with the CE symbol.
Die oben genannten medizinischen Produkte sind mit dem CE-Zeichen gekennzeichnet.

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#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
26.	PROGESTERONE	K207	Progesterone EIA	**DE/CA64/00115869**
27.	ESTRADIOL	K208	Estradiol EIA	**DE/CA64/00115870**
28.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K209	Testosterone EIA	**DE/CA64/00115871**
29.	CORTISOL	K210	Cortisol EIA	**DE/CA64/00115872**
30.	TRIIODOTHYRONINE	K211	T3 EIA	**DE/CA64/00115873**
31.	THYROXINE	K212	T4 EIA	**DE/CA64/00115874**
32.	FREE TRIIODOTHYRONINE	K213	fT3 EIA	**DE/CA64/00115875**
33.	FREE THYROXINE	K214	fT4 EIA	**DE/CA64/00115876**
34.	DEHYDRO-EPIANDROSTERONE SULPHATE (INCL. DHEA)	K215	DHEAS EIA	**DE/CA64/00115877**
35.	17 OH PROGESTERONE	K217	17-OH-progesterone EIA	**DE/CA64/00115878**
36.	ESTRIOL	K218	free Estriol EIA	**DE/CA64/00115880**
37.	TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE)	K219	free Testosterone EIA	**DE/CA64/00115881**
38.	CANCER ANTIGEN 125	K222	CA 125 EIA	**DE/CA64/00115882**
39.	CANCER ANTIGEN 19-9	K223	CA 19-9 EIA	**DE/CA64/00115883**
40.	CARCINOEMBRYONIC ANTIGEN	K224	CEA EIA	**DE/CA64/00115884**
41.	ALPHAFETOPROTEIN	K225	AFP EIA	**DE/CA64/00115885**
42.	CANCER ANTIGEN 15-3	K226	CA 15-3 (M12) EIA	**DE/CA64/00115886**
43.	OTHER OTHER TUMOUR MARKERS	K232	Thyroglobulin EIA	**DE/CA64/00115887**
44.	ß HUMAN CHORIONIC GONADOTROPIN (INCL. SUBUNIT)	K235	free β-HCG EIA	**DE/CA64/00115888**
45.	CYFRA 21-1	K236	CYFRA 21-1 EIA	**DE/CA64/00115889**
46.	SQUAMOUS CELL CARCINOMA ANTIGEN	K237	SCC (A) EIA	**DE/CA64/00115890**
47.	PREGNANCY ASSOCIATED PLASMA PROTEIN - A (DOWNS)	K238	PAPP-A EIA	**DE/CA64/00115892**
48.	OTHER OTHER TUMOUR MARKERS	K239	HE4 EIA	**DE/CA64/00115893**
49.	CANCER ANTIGEN 242	K243	CA242 EIA	**DE/CA64/00115894**
50.	OTHER PREGNANCY TESTING HORMONES	K245	AMH EIA	**DE/CA64/00115896**

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#	Nomenclature term Nomenklaturbezeichnung	Catalog No. Katalog-Nr.	Name of device Produktbezeichnung	DMIDS Registration number Registriernummer
51.	HUMAN PLACENTAL LACTOGEN HPL	K246	Placental lactogen EIA	DE/CA64/00115897
52.	C-REACTIVE PROTEIN	K250	CRP EIA	DE/CA64/00115898
53.	C-PEPTIDE	K267C	C-peptide EIA	DE/CA64/00115900
54.	INSULIN	K267N	Insulin EIA	DE/CA64/00115901
55.	SEX HORMONE BINDING GLOBULIN	K268	SHBG EIA	DE/CA64/00115902
56.	TROPONIN (T + I)	K291	Troponin I EIA	DE/CA64/00115903
57.	LYME ANTIBODY IGG	K118G	Borelia burgdorferi IgG EIA	DE/CA64/00115904
58.	LYME ANTIBODY IGM	K118M	Borelia burgdorferi IgM EIA	DE/CA64/00115905
59.	EBV ANTIBODIES	K108V K108VM K108N	Epstein-Barr virus VCA IgG EIA Epstein-Barr virus VCA IgM EIA Epstein-Barr virus EBNA IgG EIA	DE/CA64/00115906

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Beata Rozwadowska * Polmed.de * Fichtenstr. 12a, 90763 Fürth, Germany

Date: **March 07, 2023**

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UkrMedCert

8O047
Сертифікація
систем менеджменту

CERTIFICATE

on compliance of Quality Management System

Registration Date:
August 02, 2024
No. UA.SM.214-21
Expiry Date: August 01, 2027
First edition: August 04, 2021

THIS IS TO CERTIFY THAT
QUALITY MANAGEMENT SYSTEM CONCERNING

The Design and Development, Manufacture, Storage and Distribution medical devices for in vitro diagnostics

was implemented by: XEMA LLC
at the address: Akademika Yefremova St. 23, Kyiv, Ukraine, 03179

meets the requirements of DSTU EN ISO 13485:2018 (EN ISO 13485:2016, IDT; ISO 13485:2016, IDT); ISO 13485:2016.

Compliance control of the certified quality management system with the requirements of the specified standard is carried out through supervision, the frequency and procedures of which are regulated by the procedures of the conformity assessment body.

The conformity assessment body UKRMEDCERT LLC, address: str. Drahomanova, building 1-A, office 2, Kyiv, 02059, Ukraine, phone: +38-067-595-02-30, https://ukrmedcert.org.ua

Head of CAB **Tetiana SUKHENKO**

The validity of a certificate of compliance can be verified in the online Register https://ukrmedcert.org.ua or by phone +38-067-595-02-30.
The original version of this Certificate is issued in Ukrainian.

Instruction for use
A solid-phase enzyme immunoassay kit
for the qualitative detection of
total antibodies (IgG, IgA, IgM) to
Giardia lamblia
in human serum or plasma

anti-Giardia lamblia EIA

Catalogue number REF **K171**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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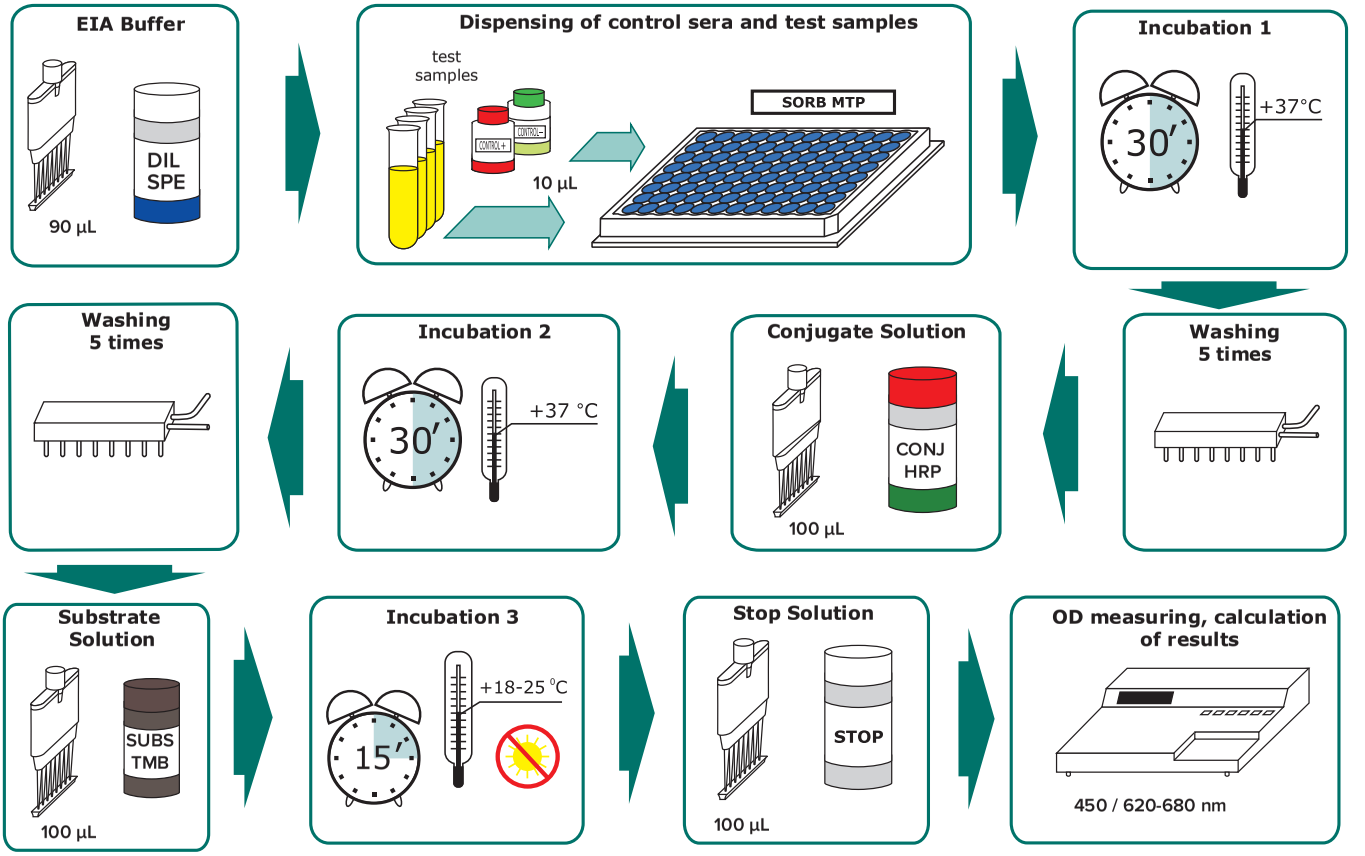
ASSAY PROCEDURE



During performing several independent series of tests, Positive and Negative Control Serum should be used **each time**.

K171

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SAMPLES IDENTIFICATION PLAN 10

Instruction for use
A solid-phase enzyme immunoassay kit for the qualitative detection of total antibodies (IgG, IgA, IgM) to *Giardia lamblia* in human serum or plasma
anti-Giardia lamblia EIA

1. INTENDED USE

The anti-Giardia lamblia EIA kit is an enzyme immunoassay, intended for the qualitative detection of total antibodies (IgG, IgA, IgM) to *Giardia lamblia* in human serum or plasma.

The field of application is clinical laboratory diagnostics.

2. GENERAL INFORMATION

Giardia lamblia (intestinalis) causes giardiasis (giardiasis), a parasitic infection that occurs in the form of latent parasitism and manifest forms (intestinal dysfunction). The causative agent of giardiasis is widespread, especially in regions with bad sanitary culture. The main mechanism of *Giardia lamblia* infection is faecal-oral. The disease is common among all age groups, however, the main contingent is preschool children. Vegetative forms of the parasite exist only on the surface of the mucous membrane of the upper small intestine. This leads to the fact that Giardia mechanically blocks the mucous membrane and disrupts parietal digestion and motor activity of the small intestine. Giardia causes impaired absorption of fats, carbohydrates, vitamins C and B12. It is worth noting that due to its susceptibility to bile acids, *Giardia lamblia* cannot directly cause liver disease and cholecystocholangitis, but it causes secondary bacterial infection (due to reflex biliary dyskinesia). Symptoms of giardiasis may include diarrhoea, fatigue, swelling, apathy, weight loss, decreased appetite, pallor, and muscle cramps. In the gastrointestinal tract, giardiasis manifests itself mainly in the form of enterocolitis with catarrhal manifestations. The diagnosis of infection caused by *Giardia lamblia* is carried out by microscopic or culture methods, as well as by determining specific antibodies in the blood serum.

3. TEST PRINCIPLE

The determination of total antibodies (IgG, IgA, IgM) to *Giardia lamblia* is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigens *Giardia lamblia*. Second antibodies – a blend of anti-species (specific to IgG, IgM, IgA) monoclonal antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to *Giardia lamblia* antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated anti-species monoclonal antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3’,5,5’-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured specific antibodies to *Giardia lamblia* in test specimen.

4. KIT COMPONENTS

Code of component	Symbol	Name	Volume	Qty, pcs.	Description
P171Z	SORB MTP	**Microplate**	-	1	96-well polystyrene strip microplate coated with recombinant antigen *Giardia lamblia*, ready to use
CN171Z	CONTROL -	**Negative Control Serum K-**	0.5 mL	1	Solution based on human serum, free of specific antibodies to *Giardia lamblia*, with preservative, ready to use (yellow liquid)
CP171Z	CONTROL +	**Positive Control Serum K+**	0.2 mL	1	Solution based on human serum pool with a high content of specific antibodies to *Giardia lamblia*, with preservative, ready to use (red liquid)
T171Z	CONJ HRP	**Conjugate Solution**	12 mL	1	Solution of a blend of anti-species (specific to IgG, IgM, IgA) monoclonal antibodies conjugated with horseradish peroxidase, ready to use (green liquid)
SP171Z	DIL SPE	**EIA Buffer**	12 mL	1	Buffer solution with detergent and preservative, ready to use (purple liquid)
R055Z	SUBS TMB	**Substrate Solution**	12 mL	1	Tetramethylbenzidine (TMB) substrate solution, ready to use (colourless liquid)
S008Z	BUF WASH 26X	**26x Concentrate Washing Solution**	30 mL	1	Buffer solution with detergent, 26x concentrate (colourless liquid)
R050Z	STOP	**Stop Solution**	12 mL	1	5.0% solution of sulphuric acid, ready to use (colourless liquid)
The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.).					

5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450/620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

6. WARNING AND PRECAUTIONS

In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The anti-Giardia lamblia EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The anti-Giardia lamblia EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- the remaining strips should be immediately resealed in the bag along with the silica gel, closed with the zip-lock, and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, EIA Buffer and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution and Control Serums after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. The remaining strips should be immediately resealed in the bag along with the silica gel and closed with the zip-lock to prevent moisture from affecting the plate's strips.

9.3. Washing Solution preparation

Add the contents of the 30 mL Washing Solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

Quantity of strips	1	2	3	4	5	6	7	8	9	10	11	12
Volume of the Washing Solution concentrate, mL	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25	27.5	30
Volume of water, mL	62.5	125	187.5	250	312.5	375	437.5	500	562.5	625	687.5	750

10. ASSAY PROCEDURE

10.1. Put the desired number of strips into the frame based on the number of test samples and 4 wells for Positive and Negative Control Serum (1 well for Positive Control (CP) and 3 wells for Negative Control Serum (CN)).

10.2. Dispense **90 µL of EIA Buffer** to all wells.

10.3. Dispense **10 µL of Positive and Negative Control Serum as well as 10 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Positive and Negative Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.
Attention! When adding blood serum (plasma) samples, the colour of the solution changes.

During performing several independent series of tests, Positive and Negative Control Serum should be used each time.

Scheme of introduction of samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	CP	SAMP5	SAMP13	SAMP21								
B	CN	SAMP6	SAMP14	SAMP22								
C	CN	SAMP7	SAMP15	SAMP23								
D	CN	SAMP8	SAMP16									
E	SAMP1	SAMP9	SAMP17									
F	SAMP2	SAMP10	SAMP18									
G	SAMP3	SAMP11	SAMP19									
H	SAMP4	SAMP12	SAMP20									

10.4. Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5. At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well **5 times** using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution in the wells after each aspiration or decantation should be no more than 5 µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6. Add **100 µL of Conjugate Solution** to all wells.

10.7. Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8. At the end of the incubation period, aspirate and wash each well **5 times** as described in 10.5.

10.9. Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.10. Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11. Read the optical density (OD) of the wells at 450 nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution.

11. TEST VALIDITY AND CALCULATION OF RESULTS

11.1. The test results are valid only if Positive and Negative Control Serum are within the specified ranges and if all other test parameters are also within the given assay specifications, namely:

- OD of Negative Control Serum < 0.15;
- OD of Positive Control Serum > 0.30.

11.2. Calculate the mean OD value of the Negative Control Serum:

meanOD(CN) = (OD1(CN) + OD2(CN) + OD3(CN))/3

If one of the OD values of the Negative Control Serum differs significantly, it should be discarded and the meanOD(CN) should be calculated using the remaining OD values of the Negative Control Serum.

11.3. Calculate the Cut off value by adding to the mean OD value of the Negative Control Serum the coeficient 0.2.

Cut off = meanOD(CN) + 0.2

11.4. Calculate Positivity Index (PI) for each sample by dividing the OD of the sample by Cut off value:

PI = ODsample/Cut off

12. INTERPRETATION OF THE RESULTS

If PI value > 1.1 the result is **POSITIVE**,
If PI value is between 0.9 and 1.1 the result is **EQUIVOCAL**,
If PI value < 0.9 the result is **NEGATIVE**.

If equivocal results are obtained, it is recommended to retest the sample in several replicates. If the result is equivocal again, a new sample should be obtained within 5-7 days and retested.

If the result remains equivocal, the sample should be considered negative.

13. PERFORMANCE CHARACTERISTICS

13.1. Analytical performance characteristics

13.1.1. Precision of Measurement

Repeatability (Intra assay repeatability) was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

No. sample	mean PI	CV, %
1	3.4	2.9
2	9.1	4.8

Reproducibility (Inter assay reproducibility) was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

No. sample	mean PI	CV, %
1	3.36	2.8
2	9.07	5.0

13.1.2. Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

13.2. Diagnostic performance characteristics

The clinical sensitivity and specificity of the assay were evaluated on a sample of 89 positive and 39 negative clinical serum samples and are 97.8% and 97.4%, respectively. The positive predictive value (PPV) of the reagent kit is 98.9% and the negative predictive value (NPV) is 95%. The relative specificity of the assay was studied in a sample of 92 donor sera characterised for the absence of antibodies to Giardia lamblia in commercial kits and was 98.9%.

14. LIMITATIONS

A positive test result indicates that the patient has antibodies specific to *Giardia lamblia* antigens. The diagnosis cannot be made on the basis of presense antibodies to *Giardia lamblia* alone and requires confirmation, including assessment of the patient's clinical presentation and history.

A negative result indicates the absence of antibodies to *Giardia lamblia* or antibody levels below the limit of sensitivity of the kit.

The results of serum tests in patients with immunosuppression and immunological disorders should be interpreted with caution.

15. REFERENCES

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SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

Instruction version/date: 2024.07

K171IE

SAMPLES IDENTIFICATION PLAN

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

LOT _____ DATE _____

Instruction version/date: 2024.07

	Manufacturer
IVD	*In vitro* diagnistic medical device
REF	Catalogue number
	Use-by date
LOT	Batch code
	Temperature limit
	Contains sufficient for <n> tests
	Caution
	Consult instructions for use
	Conformity Marking with technical regulations in Ukraine
EC REP	Authorized representative in the European Community/European Union
CE	CE Conformity Marking

For any issues related to operation of the kit and technical support, please contact by telefon number

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