BLACKBOX® LASV IgM ELISA Kit (Lassa Virus, IgM)

For Research Use Only (RUO)

REF: ELM.004

Lot No. (See product label)

Size: 96-wells

Principle: µ-capture ELISA

Type: Qualitative

Storage: Conjugate has to be stored at -20°C. Store all other kit components at 2-8°C.

Intended Use

The BLACKBOX® LASV IgM ELISA Kit is intended for qualitative detection of IgM antibodies to LASV in human serum.

In conjunction with the BLACKBOX® LASV IgG ELISA Kit, the assay provides serological evidence of an acute or past infection with LASV. Test results have to be critically assessed with reference to clinical symptoms, available anamnestic information and the results of other diagnostic tests performed.

The kit is not intended for self-testing. Assay performance characteristics have not been established for automated instruments.

General Description: Lassa Fever

Lassa fever is an infectious disease endemic in several West African countries including Guinea, Liberia, Nigeria, and Sierra Leone [1]. The causative agent for this zoonotic disease is a virus of the *Arenaviridae* family, the Lassa Virus (LASV). Virus transmission to humans occurs either by inhalation or ingestion of excrements of the natural reservoir host, the multimammate rat *Mastomys natalensis*, or by direct contact with body fluids from infected patients. In about 20% of infected individuals, symptoms like fever, weakness, headache, sore throat, cough, nausea, vomiting, diarrhoea and muscle, abdominal and chest pain develop after an incubation period of 6 – 21 days; approximately 80% of infections remain asymptomatic [1]. Due to the severity of the disease, the limited therapeutic options and the high risk of human to human transmission, LASV is classified as a virus of the highest biological risk class (Biosafety Level 4).
LASV-specific IgM antibodies usually become detectable towards the end of the first week post onset of symptoms and may persist for months to years [1]. LASV-specific IgG antibodies emerge shortly after or concurrently with LASV-specific IgM antibodies [2] and may persist for decades [1].

Test Principle

The BLACKBOX® LASV IgM ELISA Kit is based on the µ-capture ELISA principle. Diluted human control sera and patient serum samples are applied to a microwell plate coated with anti-human IgM antibodies. In a subsequent incubation step (preceeded by plate washing to remove unbound serum components), HRP (horseradish peroxidase) - labeled recombinant LASV antigen is applied to the plate. In case of LASV-IgM positive sample material, the HRP-labeled antigen is specifically bound to the microtiter plate well, where it can be detected (after washing away unbound material) by application of the colorimetric HRP substrate TMB. After stopping the enzymatic reaction, the assay result is generated by measuring the optical density of the solution in the well at 450/620 nm.

Reagents and materials provided in the kit

Component	Supplied amount/ packaging	Color coding	Storage
Microwell Plate (IgM)	12 strips in sealed aluminium pouch with desiccant bag	n.a.	2°C - 8°C
Positive Control (Ready To Use)	700 µl in 2.0 ml vial	red cap	2°C - 8°C
Negative Control (Ready To Use)	1400 µl in 2.0 ml vial	white cap	2°C - 8°C
Sample Dilution Buffer (SDB)	100 ml in 125 ml bottle	clear cap	2°C - 8°C
Conjugate Dilution Buffer (CDB)	14 ml in 15 ml bottle	blue cap	2°C - 8°C
10X Wash Buffer	100 ml in 125 ml bottle	clear cap	2°C - 8°C
Conjugate (HRP-labelled recombinant LASV antigen)	25 µl in 0.5 ml vial	blue cap	-20°C
Substrate - TMB	14 ml in 15 ml amber bottle	amber cap	2°C - 8°C
Stop Solution	14 ml in 15 ml bottle	clear cap	2°C - 8°C
Adhesive foil	2 pieces	n.a.	n.a.
Instruction for Use	1	n.a.	n.a.

Table 1. Reagents and materials provided in the kit.

The kit allows the performance of 96 reactions, including positive and negative controls. For analysis of small numbers of patient samples, provided reagents are sufficient for 12 independent tests (1 strip per test, 5 patient samples per strip).

For information on reagents' shelf life and handling/security instructions see page 6 of this manual.

Materials/instruments required but not supplied in the kit

For preparation of 1X Wash Buffer:
Deionized water
Graduated cylinder

Pipetboy
Glass or plastic pipettes for volumes up to 25 ml
For preparation of other reagents/serum samples and assay performance:
Pipettes for volumes up to 10 µl, 100/200 µl and 1000 µl
Pipette tips for volumes up to 10 µl (short, graduated tips are recommended), 100/200 µl and 1000 µl
Microcentrifuge tubes
Paper towels/absorbent paper
Timer
ELISA plate reader (450 nm, 620 nm)

Optional:
Eight-channel pipette
Reagent reservoirs
Dispenser pipette and tips
Microplates with V bottom
Automated ELISA plate washer
Incubator set to 23°C

Specimen collection, preparation, storage and handling

The BLACKBOX® LASV IgM ELISA Kit has been developed using human sera. Assay performance was not tested using whole blood, plasma or other specimens. Use of hyper-lipemic, hemolyzed, icteric or contaminated sera may cause erroneous results.

For serum preparation, blood samples have to be collected by approved venipuncture procedures by qualified personnel using appropriate collection tubes allowing blood clotting. For clotting, incubate the blood sample for 30 min at RT (alternatively: overnight at 4°C). After centrifugation (1400 x g, 10 min, 4°C), aseptically transfer the supernatant (= serum) to a fresh sterile tube.

Serum samples can be kept at RT for short periods of time (< 8 hours). For storage, serum samples should be refrigerated (4°C, < 6 months) or frozen (-20°C or -80°C, long term storage). Repeated freeze/thaw cycles should be avoided. It is recommended to ship serum samples on dry ice. After thawing, serum samples have to be mixed gently but thoroughly.

If inactivation is necessary, please apply the following protocol:
Prepare a 2% Triton X-100/PBS solution (not supplied) and mix it 1:1 with the sera. Incubate at 37°C for 1 hour. Please note, as this dilutes the sera in half, twice the volume of these inactivated sera will need to be used when preparing the sample dilutions for the assay to achieve the correct dilution factor, e.g. mix 20 µl inactivated serum + 480 µl SDB.

Test Procedure

General remarks

- Perform all pipetting steps at room temperature (20°C – 25°C) using calibrated, well maintained pipettes and strictly follow the ELISA procedure protocol described below. Deviations in assay parameters like volumes, incubation times and incubation temperatures may cause invalid results.

- Mix all reagents gently but thoroughly before use.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- To prevent condensation, the microwell plate (sealed in aluminium pouch with desiccant bag) has to be equilibrated to room temperature (20°C – 25°C) at least 30 minutes before opening the package to remove the required number of microwell strips. Unused microwell strips can be stored in presence of the desiccant bag at 4°C in the resealed aluminium pouch.
- Upon arrival, **store the conjugate at -20°C**. For conjugate dilution (see below) remove the necessary amount of conjugate from the vial and immediately place the residual conjugate back in the freezer. Due to the addition of glycerol to the conjugate storage buffer, it is not necessary to thaw the conjugate solution before pipetting.
- All other reagents (SDB, CDB, Wash Buffer, TMB substrate, Stop solution) should be equilibrated to room temperature (20°C – 25°C) before use.
- Plate washing can be performed manually using a multi-channel pipette. Preferably, an automated plate washer can be used. In both cases, quantitative removal of wash solution after the washing steps is mandatory. Remaining buffer can be removed by tapping the microplate face-down on an absorbent paper towel. **Important: When using an automated plate washer, account for the additional volume needed for system priming (not included in Table 2 below) when calculating the required volume of 1 x Wash Buffer.**
- Avoid cross-contamination of wells during all pipetting and washing steps.
- Avoid the formation of air bubbles during all pipetting steps. Especially air bubbles present during the OD measurement may cause false readings. If air bubbles do occur during the development step, these can be burst before OD measurement by carefully touching them with a dry, fresh pipette tip.

Preparation of reagents and specimen

- **1X Wash Buffer.** Wash Buffer is provided in the kit as a 10X stock solution. In case of salt precipitate having formed in the stock solution, the solution has to be warmed up to approximately 30°C – 40°C to completely dissolve the precipitate. To obtain 1X Wash Buffer, dilute the required amount of 10X Wash Buffer stock solution in deionized water (volumes depending on number of microwell strips used, see Table 2 below).
- **Conjugate dilution.** Dilute the conjugate stock according to table 2. The conjugate is provided in a viscous storage buffer containing 50% glycerol. Thus, pipette the stock solution carefully under visual control and make sure that no additional solution is attached to the outside of the pipette tip. Make sure that the conjugate stock solution is transferred quantitatively to the CDB by pipetting up and down several times. Always prepare a fresh conjugate working dilution before performing the test and discard residual working dilution afterwards.
- **Control samples dilution.** The control samples are supplied Ready To Use and should not be diluted.
- **Serum samples dilution**. Dilute the serum samples in SDB (100 µl SDB + 1 µl serum).
- **TMB substrate, Stop Solution.** Both solutions are provided in the kit ready to use (required volume depending on the number of microwell strips used, see Table 2 below).

# strips	Wash Buffer		Conjugate working dilution		TMB (ml)	Stop solution (ml)
	10 x Wash Buffer (ml)	Deionized water (ml)	Conjugate stock (µl)	CDB (µl)		
1	2	18	1	499	1	1
2	4	36	2	998	2	2
3	6	54	3	1497	3	3
4	8	72	4	1996	4	4
5	10	90	5	2495	5	5
6	12	108	6	2994	6	6
7	14	126	7	3493	7	7
8	16	144	8	3992	8	8
9	18	162	9	4491	9	9
10	20	180	10	4990	10	10
11	22	198	11	5489	11	11
12	24	216	12	5988	12	12

Table 2: Preparation of reagents for different numbers of microwell strips used for testing.

ELISA procedure

1. ***Plate and reagents preparation.*** Prepare required number of microwell strips, 1X Wash Buffer, conjugate dilution and serum samples dilutions as stated above in section "Preparation of reagents and specimen".
2. ***Pipetting of control samples and serum samples***. Pipette 50 µl of Ready To Use control samples (1 well positive control, 2 wells negative control) and diluted serum samples into the respective wells. Make sure that the bottom of the wells is completely covered by gently tapping the plate on the desktop.
3. ***Serum incubation***. Seal strips with adhesive foil and incubate the strips for 60 min at room temperature (20°C – 25°C).
4. ***Plate washing.*** Wash strips 3 times with 300 µl 1X Wash Buffer per well. Soak strips for 30 sec in between washes. Remove residual Wash Buffer by tapping the plate upside-down on paper towels and then, without letting the wells dry out, immediately proceed with step 5.
5. ***Pipetting of conjugate.*** Pipette 50 µl of conjugate working solution into each well. Make sure that the bottom of the wells is completely covered by gently tapping the plate on the desktop.
6. ***Conjugate incubation***. Seal plate using the adhesive foil provided with the kit and incubate for 60 min at room temperature (20°C – 25°C).
7. ***Plate washing.*** Wash strips 3 times with 300 µl 1X Wash Buffer per well, allowing the wells to soak for 30 sec with each addition of 1X Wash Buffer before aspiration/removal of the buffer. Remove residual Wash Buffer by tapping the plate upside-down on paper towels and then, without letting the wells dry out, immediately proceed with step 8.
8. ***Substrate incubation.*** Add 100 µl TMB substrate to each well; incubate 20 min at room temperature in the dark. Wells generating a positive result will turn blue.
9. ***Stopping.*** Add 100 µl of Stop Solution to each well. The blue color of wells generating a positive result will turn yellow.
10. ***Measurement.*** Measure optical density at 450 nm and 620 nm using a microplate reader within 30 min after stopping the assay. Calculate the difference OD450 – OD620.

Evaluation of results

Test results can be considered valid if the following criteria are met:
OD450-OD620 of positive control > 90% of the value showed on the batch-specific Certificate of Analysis.
OD450-OD620 of negative control < 0.1

Use the average absolute OD450-OD620 absorbance value for the negative control sample ($OD_{neg, av}$) to calculate the assay cut-off OD_{CO} according to the following formula:

$OD_{neg, av} + 0.150 = OD_{CO}$

The Index Values IV for the tested serum samples can then be calculated according to:

$IV_{Sample} = OD450\text{-}OD620(sample)/OD_{CO}$

Evaluate the obtained results according to Table 3.

Index value IV	Result LASV IgM	Interpretation
$IV_{Sample} > 1.1$	positive	Indicative of recent or current infection with LASV, assess positive result by additional testing and/or clinical findings.
$0.9 < IV_{Sample} < 1.1$	equivocal	Ambiguous result, repeat test for this serum and (if available) a follow-up sample of the patient taken 3-5 days later and/or analyze sample by additional testing.
$IV_{Sample} < 0.9$	negative	No anti-LASV IgM antibodies were detected. This finding does not necessarily exclude an acute infection with LASV, because IgM antibodies usually are not detectable before day 5 - 7 after onset of illness. If available, a follow-up sample of the patient taken several days later should be tested.

Table 3: Result classification for index values and interpretation of results.

Kit shelf life and kit component storage

- Under correct storage conditions (see below), stability of the kit is guaranteed until the expiration date label on the box. Do not use the kit after the expiration date.
- Upon receipt of the kit**, immediately freeze the Conjugate (recombinant HRP-labelled LASV antigen) stock solution at -20°C**. For Conjugate dilution remove the necessary amount of Conjugate from the vial and immediately place the residual Conjugate back in the freezer. Due to the addition of glycerol to the Conjugate storage buffer, it is not necessary to thaw the Conjugate solution before pipetting.

- All other kit components have to be stored refrigerated (+2°C to +8°C).

General safety information

- The kit is intended for research use only (RUO).
- Tests have to be performed by qualified laboratory personnel.
- Wear appropriate protective clothing (lab coat, gloves, goggles) when handling kit components and patient sera.
- As blood products, positive and negative control samples as well as patient samples should be treated as potentially infectious. It is recommended to handle and dispose of samples and all material having been in contact with the samples (i.e. pipette tips, tubes, microwell strips) under appropriate safety conditions. With the LASV being a virus of biological safety level 4, inactivation of patient sera prior to testing is advised. Inactivation using Triton X-100 is recommended, as described above. **Do not use heat inactivation.**
- The Stop Solution contains sulfuric acid which is corrosive. Contact with skin or eyes must be avoided. In case of exposure, flush with large amounts of water.

Reference List

[1] Raabe V, Koehler J. (2017). Laboratory diagnosis of Lassa fever.
J Clin Microbiol 55:1629 –163.

[2] Gabriel M, Adomeh DI, Ehimuan J, Oyakhilome J, Omomoh EO, Ighodalo Y, Olokor T, Bonney K, Pahlmann M, Emmerich P, Lelke M, Brunotte L, Ölschläger S, Thomé-Bolduan C, Becker-Ziaja B, Busch C, Odia I, Ogbaini-Emovon E, Okokhere PO, Okogbenin SA, Akpede GO, Schmitz H, Asogun DA, Günther S (2018). Development and evaluation of antibody-capture immunoassays for detection of Lassa virus nucleoprotein-specific immunoglobulin M and G.
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