ReLASV® Pan-Lassa NP IgG ELISA Kit

(Human anti-LASV NP Antibody)

For Research Use Only

Not for use in diagnostic procedures.
The performance characteristics of this product have not been established.

INSTRUCTIONS FOR USE

PRINCIPLE OF THE TEST

Lassa fever (LF) is a severe, often fatal, febrile illness endemic to West Africa caused by Lassa virus (LASV; family Arenaviridae)(*1, 2*). LASV encodes four major proteins, including the envelope glycoproteins (GP1 and GP2), the structural protein Z and the nucleoprotein (NP). Advanced protein chemistry techniques have been used to develop non-infectious, recombinant LASV NP antigen(*3*). The ELISA utilizes a mixture of LASV NP antigen from the three most prevalent lineages of LASV (lineage II, III in Nigeria, lineage IV in Sierra Leone, Guinea, Liberia, and Mali) to provide Pan-Lassa cross-reactivity. The ReLASV Pan-Lassa NP IgG ELISA Kit has demonstrated capacity to detect LASV-specific IgG antibody in active LF cases and LF survivors (*4-9*).

This assay is a direct ELISA detecting Human IgG antibody specific for LASV NP. Diluted samples, Calibrator, Positive Control, and Negative Control are incubated in microwells coated with a mixture of recombinant LASV NP antigens. Incubation allows the anti-LASV antibody present in the samples to react with the immobilized antigen mixture. After the removal of unbound serum or plasma proteins by washing, anti-human IgG antibodies labeled with horseradish peroxidase (HRP) are added, forming complexes with the bound IgG anti-LASV antibody. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of TMB substrate. Color develops in the wells at an intensity proportional to the concentration of IgG anti-LASV antibody in the sample.

Optical Density (O.D.) results are obtained by reading the absorbance at 450nm (minus 620 - 650nm reference) using an ELISA plate reader. It is recommended that the user establish a cut-off for the study population using LASV sero-negative samples. It is also recommended that LF IgG positive convalescent LF sample from the study population be included in each assay as an additional reference sample.

REAGENTS

Store at 2–8°C. Do Not Freeze. Each ReLASV® Pan-Lassa NP IgG ELISA Kit contains the following reagents:

Component	2-plate kit (10200)	10-plate kit (10200-10)
NP Antigen Coated Microwell Plate (resealable bag with desiccant)	Two 12x8 plates	Ten 12x8 plates
Anti-Hu IgG HRP Conjugate Solution (Blue)	1 x 25 mL bottle	1 x 120 mL bottle
Anti-NP IgG Calibrator (recombinant human MAb in human plasma, lyophilized)	2 x 0.25 mL vials	10 x 0.25 mL vials
Anti-NP IgG Positive Control (recombinant human MAb in human plasma, lyophilized)	2 x 0.25 mL vials	10 x 0.25 mL vials
Negative Control (human plasma, lyophilized)	2 x 0.25 mL vials	10 x 0.25 mL vials
Sample Diluent 2	1 x 120 mL bottle	3 x 250 mL bottles
ELISA Wash Concentrate (33X PBS/Tween 20).	1 x 120 mL bottle	2 x 120 mL bottles
ELISA One-Component Substrate (TMB and H_2O_2); ready to use. (Amber Bottle)	1 x 25 mL bottle	1 x 120 mL bottle
ELISA Stop Solution (2% methanesulfonic acid). (Red Cap)	1 x 25 mL bottle	1 x 120 mL bottle

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

Lassa Virus is classified as NIAID Category A agent. Handling of infectious blood and serum requires advanced biocontainment (BSL-4) facilities. Use of this product in BSL -1, -2 or -3 facilities is not recommended. If advanced biocontainment facilities are not available, the use of all possible universal precautions is highly recommended including, face shields, masks or respiratory equipment, disposable gowning, and gloves. Decontamination equipment and solutions should be readily available. Biohazardous wastes should be autoclaved and/or incinerated.

1. Human source material used to prepare the controls included in this kit has been tested and shown to be nonreactive for hepatitis B surface antigen, negative for antibodies to HIV, and negative for HCV. However, all human blood derivatives, including patient samples, should be handled as potentially infectious material (Universal Precautions).
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
4. When testing in facilities with limited biocontainment equipment, wear disposable gloves while handling samples and kit reagents and wash hands thoroughly afterwards.
5. When testing in facilities with limited biocontainment equipment, wear disposable face shields, masks and gowning while handling samples and kit reagents and dispose in biohazard waste containers after use.
6. When testing in facilities with limited biocontainment equipment, wear rubber boots while handling samples and kit reagents and decontaminate with bleach solution after use.
7. One-component substrate can cause irritation to the eyes and skin. Absorption through the skin is possible. Use gloves when handling substrate and wash thoroughly after handling. Avoid mixing with oxidizing agents.
8. Certain components are labeled with the following: Irritating to eyes (R 36). Avoid contact with skin and eyes (S 24/25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26). If swallowed, seek medical advice immediately and show container or label (S 46).
   Irritant  . Biological Risk  .

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA) are the preferred sample matrices. Blood should be collected by venipuncture, and the serum separated from the cells by centrifugation after clot formation. If not tested immediately, specimens should be stored at 2–8°C. If specimens are to be stored for more than 72 hours, they should be frozen at –20°C or below. Avoid repeated freezing and thawing. Do not use grossly hemolyzed, icteric, or lipemic serum as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

If plasma (EDTA) is to be used, blood should be collected by venipuncture and the plasma separated from the cells immediately by centrifugation following the blood tube manufacturers recommendations. The supernatant must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable in order to minimize platelet contamination. If not tested immediately, plasma samples should be stored as described for serum.

INSTRUCTIONS FOR USE

MATERIALS PROVIDED:

ReLASV® Pan-Lassa NP IgG ELISA Kit; see “Reagents,” for a complete listing.

MATERIALS REQUIRED BUT NOT PROVIDED:

- Laboratory grade water
- Graduated cylinders
- Precision pipettors capable of delivering between 10 µL and 1000 µL, with appropriate tips
- Sample dilution tubes or deep-well sample dilution plates
- Reagent reservoirs
- Miscellaneous glassware appropriate for small volume handling
- Flask or bottle, 1 liter
- Disposable gloves
- Plate reading spectrophotometer capable of reading absorbance at 450nm (with a 620 - 650nm reference if available)
- Multichannel pipettors capable of delivering to 8 or 12 wells simultaneously

PROCEDURAL NOTES

1. Bring samples and kit reagents to ambient temperature (18–30°C) and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
2. The plate reader should be programmed for reagent blank subtraction.
3. Good washing technique is critical for optimal performance of the assay. An automated microtiter plate washing system should be used be used with bleach added to the waste reservoir.
4. IMPORTANT: Failure to adequately remove residual Wash Solution can cause inconsistent color development of the Substrate.
5. Use a multichannel pipettor capable of delivering to 8 or 12 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.
6. Carefully controlled timing of all steps is critical. All calibrator dilutions, controls and samples must be added within a five-minute period. Batch size of samples should not be larger than the amount that can be added within this time period.
7. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
9. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
10. Incubation temperatures above or below ambient temperature (18–30°C) may contribute to inaccurate results.
11. Avoid contamination of reagents when opening.
12. Do not use kit components beyond expiration date.
13. Do not use components from different kit lot numbers.

REAGENT PREPARATION

1X ELISA Wash Solution: Measure 30 mL of Wash Concentrate (33X) and dilute to 1 liter with laboratory grade water. Store unused Wash Solution in the refrigerator at 2–8°C . Discard if the solution shows signs of microbial contamination.

Lyophilized Calibrators and Controls: Reconstitute IgG Calibrator, IgG Positive Control, and Negative Control with 0.250mL laboratory grade water. Mix gently for several minutes until completely dissolved. Unused portion should be store at 2-8°C for up to 7 days or stored frozen (-20°C or less) for longer periods.

ASSAY PROCEDURE

1. Remove any microwell strips that will not be used from the frame and store them in the bag provided.
2. Prepare a five-point Anti-NP IgG Calibrator curve: Label five tubes for Calibrator 1 – 5.

In tube #1, prepare a 1:101 dilution of Calibrator in Sample Diluent (green) by adding 10 µL Calibrator to 1000 µL Sample Diluent.

Add 500 µL of Sample Diluent (green) to tubes # 2-5.

Remove 250 µL from dilution tube #1, transfer to dilution tube #2 and mix well.

Repeat this 3-fold serial dilution series through tube 5.

The value of the Calibrator is indicated on the vial label. The value of dilutions 2 – 5 are calculated by dividing the label value by each dilution factor (DF).

Example:

Dilution #	DF	Volume to Add		Sample Diluent		Calibrator value
1	-	10 µL Calibrator	+	1000 µL	=	100.0 µg/mL
2	3	250 µL Dilution #1	+	500 µL	=	33.3 µg/mL
3	9	250 µL Dilution #2	+	500 µL	=	11.1 µg/mL
4	27	250 µL Dilution #3	+	500 µL	=	3.7 µg/mL
5	81	250 µL Dilution #4	+	500 µL	=	1.2 µg/mL

3. A reagent blank control must be run in duplicate on each plate. This well is then treated the same as sample wells in subsequent assay steps.
4. Duplicate determinations are recommended. Prepare a 1:100 dilution of controls and samples in Sample Diluent; e.g., 10 µL sample added to 1000 µL Sample Diluent equals a 1:101 sample dilution.
5. Add 100 µL of prepared diluted Calibrator, Positive Control, Negative Control, diluted samples, and reagent blank to the appropriate microwells.
6. Incubate 30 minutes at ambient temperature (18-30°C).
7. After the incubation is complete, wash 4 times (300uL/well) with wash solution. Tap the plate firmly on absorbent paper to remove residual wash fluid.
8. Add 100 µL Anti-Hu IgG HRP Conjugate Solution to the wells.
9. Incubate for 30 minutes at ambient temperature (18-30°C).
10. Wash 4 x 300µL/well with wash solution as in step 7. Tap the plate firmly on absorbent paper to remove residual wash fluid. Do not allow the wells to dry out.
11. Add 100 µL One-Component Substrate to each well and incubate for 10 minutes at ambient temperature (18-30°C) while protected from light. Blue color will develop in wells with positive samples.
12. Add 100 µL Stop Solution (2% methanesulfonic acid) to each well to stop the enzyme reaction. Blue substrate will turn yellow and colorless substrate will remain colorless. Read the O.D. (i.e. absorbance) of each well at 450nm (with 620nm – 650nm reference, if available). The O.D. values should be measured within 5 minutes after the addition of Stop Solution.

RESULTS

1. Calculate the mean O.D. values for the duplicates of the Calibrator dilutions, Reagent Blank, Positive Control, Negative Control, and samples. Subtract mean O.D. 620nm – 650nm reference from mean O.D. 450nm.
2. Estimate the concentration of IgG by plotting the mean O.D. obtained for each dilution of IgG Calibrator (y axis) against the corresponding IgG Calibrator value (x axis) using curve fitting software. A 4-Parameter curve fit calculation is recommended.
3. Ensure that all quality control parameters have been met (see Quality Control) before reporting results.
4. A new Calibrator curve should be prepared with every assay.

QUALITY CONTROL

1. The mean O.D. of the reagent blank should be less than 0.150. Readings greater than 0.150 may indicate possible reagent contamination or inadequate plate washing.
2. O.D. values for duplicates of the controls or patient samples should be within 25% CV of the mean O.D. value, for samples with O.D. readings greater than 0.250.
3. The average IgG concentration of the Positive Control recovered against the Calibrator Curve should be within the range given on the vial label.
4. Each laboratory should determine their own normal range for the appropriate population.

NORMAL CUT-OFF

To be determined experimentally by the end user within a study population. Cut-off range has not been established by manufacturer.

LIMITATIONS OF THE TEST– FOR RESEARCH USE ONLY – NOT FOR USE IN DIAGNOSTIC PROCEDURES

IgG anti-LASV antibody levels obtained with this assay are not for use in diagnostic procedures.

Strain variability of Old World Arenaviruses or LCMV may affect performance of the assay.

The presence of Rheumatoid Factor (RF) in LF samples may interfere with ELISA methods by binding to antibodies. The presence of RF should be considered when evaluating results.

Testing LF samples containing excess hemoglobin, lipids, and/or bilirubin is not recommended as these substances may interfere with the results of the assay.

The performance characteristics of this assay have not been established.

REFERENCES

1. J. G. Shaffer *et al.*, Lassa Fever in Post-Conflict Sierra Leone. *PLoS Negl Trop Dis* **8**, e2748 (2014).
2. J. N. Hartnett *et al.*, Current and emerging strategies for the diagnosis, prevention, and treatment of Lassa fever. *Future Virology* **10**, 559-584 (2015).
3. L. M. Branco *et al.*, Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance. *Virol J* **5**, 74 (2008).
4. L. M. Branco *et al.*, Lassa hemorrhagic fever in a late term pregnancy from northern Sierra Leone with a positive maternal outcome: case report. *Virol J* **8**, 404 (2011).
5. L. M. Branco *et al.*, Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection. *Virol J* **8**, 478 (2011).
6. M. L. Boisen *et al.*, Multiple circulating infections can mimic the early stages of viral hemorrhagic fevers and possible human exposure to filoviruses in Sierra Leone prior to the 2014 outbreak. *Viral Immunol* **28**, 19-31 (2015).
7. M. L. Boisen *et al.*, Field validation of recombinant antigen immunoassays for diagnosis of Lassa fever. *Sci Rep* **8**, 5939 (2018).
8. N. Sogoba *et al.*, Lassa Virus Seroprevalence in Sibirilia Commune, Bougouni District, Southern Mali. *Emerg Infect Dis* **22**, 657-663 (2016).
9. D. Safronetz *et al.*, Annual Incidence of Lassa Virus Infection in Southern Mali. *Am J Trop Med Hyg* **96**, 944-946 (2017).

SYMBOL LEGEND

							
Manufacturer	Batch Code	Use by/ Expiry Date	Temperature Limitations	Irritant	Biological Risk	Catalog Number	Consult Instructions for Use (Package Insert)

WARRANTY

Zalgen Labs, LLC disclaims any implied warranty of merchantability or fitness for a particular use, and in no event shall Zalgen Labs, LLC be liable for consequential damage.

<u>For Technical or Customer Service</u>:
Phone: +1 301 720 0330, US Toll Free 1 833 482 8833
Fax: +1 301 349 1194
Email: admin@zalgenlabs.com

Zalgen Labs, LLC
20271 Goldenrod Lane, Suite 2083
Germantown, MD, USA 20876

Doc. No.: PI-50R 00
Effective: 2021-07-28