# ReLASV® Pan-Lassa Combo NP/Prefusion GP IgG/IgM ELISA Kit

## (Human anti-LASV NP and GP Antibodies)

## 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)*(1-4)*. 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*(5)* and GP antigen*(6)* which is stabilized in the prefusion conformation to enhance detection of LASV neutralizing antibodies*(7)*. The ELISA test utilizes a mixture of LASV NP and Prefusion GP antigens from the two most prevalent lineages of LASV (lineage II in Nigeria and lineage IV in Sierra Leone, Guinea, and Liberia) to provide Pan-Lassa cross-reactivity*(8)*. The family of ReLASV Pan-Lassa antigen and antibody products has demonstrated capacity to detect LASV antigen and LASV-specific IgG or IgM antibody in active LF cases and LF survivors*(9-14)*.

This Enzyme-linked Immunosorbent Assay (ELISA) detects human IgG or IgM antibody specific for LASV NP and GP antigens. Diluted plasma or serum samples, Calibrator, Positive Control, and Negative Control are incubated in microwells coated with a mixture of recombinant LASV NP and Prefusion GP antigens. Incubation allows the LASV-specific antibodies present in the samples to react with the immobilized antigen mixture. After the removal of unbound plasma or serum proteins with the PBS-Tween wash, the anti-human IgG (or IgM) - horseradish peroxidase (HRP) conjugate reagent is added to bind with the absorbed LASV-specific IgG (or IgM) 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 the LASV-specific IgG (or IgM) antibody in the sample. Substrate color development is stopped by addition of the ELISA Stop Solution.

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 well characterized, IgG and IgM positive, convalescent LF samples from the study population be included in each assay as additional reference samples.

### REAGENTS

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

| Component | 2-plate kit (10171) | 10-plate kit (10171-10) |
|---|---|---|
| NP and Prefusion GP 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-Hu IgM HRP Conjugate Solution (Red) | 1 x 25 mL bottle | 1 x 120 mL bottle |
| Anti-NP and GP IgG Calibrator (recombinant MAb in human plasma, lyophilized) | 2 x 0.25 mL vials | 10 x 0.25 mL vials |
| Anti-NP and GP IgM Calibrator (recombinant MAb in human plasma, lyophilized) | 2 x 0.25 mL vials | 10 x 0.25 mL vials |
| Anti-NP and GP IgG Positive Control (recombinant MAb in human plasma, lyophilized) | 2 x 0.25 mL vials | 10 x 0.25 mL vials |
| Anti-NP and GP IgM Positive Control (recombinant 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 |

| Component (Continued) | 2-plate kit (10171) | 10-plate kit (10171-10) |
|---|---|---|
| Sample Diluent 3 (Green) | 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).

   Biological Risk. 
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where kit reagents, samples, or assay wastes are handled.
4. When testing in facilities with limited biocontainment equipment, wear personal protective equipment (PPE) including disposable gloves, face shields, N95 masks, and gowning while handling samples and assay wastes. Dispose PPE in biohazard waste containers after use and wash hands thoroughly. If rubber boots are used, decontaminate with bleach solution after use.
5. 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.
6. 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. 

## SAMPLE COLLECTION AND PREPARATION

Plasma or serum are the preferred sample matrices. 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 plasma (supernatant) must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable to minimize platelet contamination. If not tested immediately, samples should be stored at 2–8°C. If samples are to be stored for more than 72 hours, they should be frozen at -20°C or below. Avoid repeated freezing and thawing.

If serum is to be used, the blood should be collected by venipuncture, and the serum separated from the cells by centrifugation after clot formation, following the blood tube manufacturers recommendations. If not tested immediately, serum samples should be stored as described for plasma.

Do not use grossly hemolyzed, icteric, or lipemic plasma (or serum) as these conditions may cause aberrant results. Samples containing visible particulate matter should be clarified by centrifugation before testing.

## INSTRUCTIONS FOR USE

### MATERIALS PROVIDED:

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

### MATERIALS REQUIRED BUT NOT PROVIDED:

- PPE – refer to Warnings and Precautions Section
- 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
- 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 with bleach added to the waste reservoir.
4. IMPORTANT: Failure to adequately remove residual Wash Solution can cause inconsistent color development of the Substrate Solution.
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.
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.
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 (or IgM) Calibrator, IgG (or IgM) Positive Control, and Negative Control with 0.250mL laboratory grade water. Mix gently for several minutes until completely dissolved. Unused portion should be stored at 2-8°C for up to 7 days or stored frozen (-20°C or less) for longer periods.

## ASSAY PROCEDURE

Note: Duplicate well testing of all Calibrator dilutions, Positive Control, Negative Control, Reagent Blank, and samples is recommended.

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

   In tube #1, prepare a 1:101 dilution of Calibrator in Sample Diluent 3 by adding 10 µL Calibrator to 1000 µL Sample Diluent 3.

   Add 500 uL of Sample Diluent 3 to tubes # 2-5.

   Remove 250 uL 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 Calibrator dilutions 2 - 5 are calculated by dividing Calibrator value by each Calibrator dilution factor (DF).

   Example:

| Dilution # | DF | Volume to Add | | Sample Diluent Volume | | Calibrator Value |
|---|---|---|---|---|---|---|
| 1 | - | 10 uL Calibrator | + | 1000 uL | = | 100 ug/mL |
| 2 | 3 | 250 uL Dilution #1 | + | 500 uL | = | 33.3 ug/mL |
| 3 | 9 | 250 uL Dilution #2 | + | 500 uL | = | 11.1 ug/mL |
| 4 | 27 | 250 uL Dilution #3 | + | 500 uL | = | 3.7 ug/mL |
| 5 | 81 | 250 uL Dilution #4 | + | 500 uL | = | 1.23 ug/mL |

3. A Reagent Blank control must be run in each plate. These wells are then treated the same as sample wells in subsequent assay steps.
4. Prepare a 1:101 dilution of the Positive Control, Negative Control, and samples in Sample Diluent 3, e.g., 10 µL sample added to 1000 µL Sample Diluent 3 equals a 1:101 sample dilution.
5. Add 100µL of the prepared Calibrator dilutions, Positive Control, Negative Control, Reagent Blank, and samples 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. Blot on absorbent paper to remove residual wash fluid. Do not allow the wells to dry out.
8. Add 100 µL of the Anti-Hu IgG (or IgM) HRP Conjugate Solution to each well.
9. Incubate for 30 minutes at ambient temperature (18-30°C).
10. Wash 4 x 300µL/well with wash solution as in step 7. Blot on absorbent paper to remove residual wash fluid. Do not allow the wells to dry out.
11. Add 100µL of the 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 100µL of the Stop Solution (2% methanesulfonic acid) to each well to stop the substrate color development. Blue substrate will turn yellow and colorless substrate will remain colorless.
13. The plate reader should be programmed to blank (or zero) the reader against the duplicate Reagent Blanks. Read the O.D. (i.e., absorbance) of each well at 450 nm (with 620 – 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, Positive Control, Negative Control, and samples. Subtract mean O.D. 620 – 650nm reference from mean O.D. 450nm.
2. Estimate the concentration of IgG (or IgM) by plotting the mean O.D. obtained for each IgG (or IgM) Calibrator dilution (y axis) against the corresponding IgG (or IgM) 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 (or IgM) 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

LASV-specific IgG (or IgM) antibody concentrations 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**


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## 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.

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