ReEBOV® IgG/IgM ELISA Test Kit
(Human anti-EBOV Antibody)

For Research Use Only

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

PRINCIPLE OF THE TEST

This test is a direct ELISA detecting Human IgG and/or IgM antibody specific for Ebola virus (EBOV) viral matrix protein 40 (VP40). Diluted samples, Reference, and Negative Control are incubated in microwells coated with recombinant EBOV VP40 antigen. Incubation allows the anti-EBOV antibody present in the samples to react with the immobilized antigen. After the removal of unbound serum or plasma proteins by washing, anti-human IgG (or IgM) antibodies, labeled with horseradish peroxidase (HRP), are added, forming complexes with the bound IgG (or IgM) anti-EBOV antibody. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of a solution containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) as the chromogenic substrate. Color develops in the wells at an intensity proportional to the serum concentration of IgG (or IgM) anti-EBOV antibody.

Results are obtained by reading the OD (optical density or absorbance) of each well in a spectrophotometer. A Reference non-human bioconjugate and Negative serum control are provided. It is recommended that the user establish a cut-off for the study population using at least 100 sero-negative serums. It is also recommended that IgG and IgM positive convalescent Ebola serums from the study population be included in each test as an additional reference sample.

REAGENTS

Store at 2–8°C. Do Not Freeze. Each ReEBOV® IgG/IgM ELISA Test Kit contains the following reagents:

Component	2-plate kit	10-plate kit
EBOV VP40 Antigen Coated Microwell Plate (resealable bag with desiccant)	Two 12x8 plates	Ten 12x8 plates
Sample Diluent 2 (Green)	1 bottle (120 mL)	4 bottles (250 mL)
Anti-EBOV IgG Reference (rabbit bioconjugate in human plasma, lyophilized)	4 vials (0.25 mL)	20 vials (0.25 mL)
Negative Control (human plasma, lyophilized)	4 vials (0.25 mL)	20 vials (0.25 mL)
Anti-Hu IgG HRP Conjugate Solution (Blue)	1 bottle (25 mL)	1 bottle (120 mL)
Anti-Hu IgM HRP Conjugate Solution (Red)	1 bottle (25 mL)	1 bottle (120mL)
ELISA One-Component Substrate (TMB and H_2O_2); ready to use (Amber Bottle)	1 bottle (30 mL)	1 bottle (120 mL)
ELISA Stop Solution 2 (1% methanesulfonic acid) (Red Cap)	1 bottle (30 mL)	1 bottle (120 mL)
ELISA Wash Concentrate (33X PBS/Tween 20)	1 bottle (120 mL)	2 bottles (120 mL)

WARNINGS AND PRECAUTIONS

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Ebola 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. Keep reagent away from ignition sources. Avoid contact 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 Xi. 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 at 1500g for 10 minutes. 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:
ReEBOV® IgG/IgM ELISA Test Kit; see “Reagents” for a complete listing.

Materials Required but not Provided:
- Reagent grade water to prepare Wash Solution (4 x 1L)
- Graduated cylinders
- Precision pipettors capable of delivering between 10 µL and 1000 µL, with appropriate tips
- Miscellaneous glassware appropriate for small volume handling
- Flask or bottle, 1 liter
- Wash bottles, preferably with the tip partially cut back to provide a wide stream, or an automated or semi-automated microwell plate washing system
- Disposable gloves
- Plate reading spectrophotometer capable of reading absorbance at 450 nm (with a 650 nm reference if available)
- Multichannel pipettors capable of delivering to 8 or 12 wells simultaneously

Procedural Notes

1. Bring samples and kit reagents to room 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 to air blank.
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. If manual plate washing is used, adequate washing is best accomplished by directing a forceful stream of wash solution from a plastic squeeze bottle with a wide tip into the bottom of the microwells.
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 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 normal room temperature (18–30°C) when required may contribute to inaccurate results.
11. Avoid contamination of reagents when opening and removing aliquots from the primary vials.
12. Do not use kit components beyond expiration date.
13. Do not use components from different kit lot numbers.

Reagent Preparation

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 Controls: Reconstitute Reference 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 Reference curve: Label five tubes for Reference 1 – 5.
   In tube #1, prepare a 1:100 dilution of Reference in Sample Diluent (green) by adding 10 µL reference to 990 µL Sample Diluent.
   Add 500 uL of Sample Diluent (green) 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 Reference is given on the label. The value of Reference dilutions 2 – 5 are calculated by dividing Reference value by each Reference dilution factor (DF).

   Example:

Dilution #	DF	Volume to Add		Sample Diluent		Reference Value
1	-	10 uL Reference	+	990 uL	=	10.0 (example)
2	3	250 uL Dilution #1	+	500 uL	=	3.3
3	9	250 uL Dilution #2	+	500 uL	=	1.1
4	27	250 uL Dilution #3	+	500 uL	=	0.37
5	81	250 uL Dilution #4	+	500 uL	=	0.12

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 the samples in Sample Diluent (green); e.g., 10 µL sample added to 990 µL Sample Diluent equals a 1:100 sample dilution.
5. Add 100 µL of prepared Reference, Normal Control, samples, and reagent blank to the appropriate microwells.

   Note: All prepared Reference, Negative Control, samples, and reagent blank may be applied to both microwell plates for concurrent IgG and IgM detection provided appropriate IgG and IgM conjugate is added to designated microwell plates.
6. Incubate 30 minutes at room 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.

   For manual washing, carefully invert the microwells and empty the fluid. Do not allow samples to contaminate other microwells. Each well should be completely filled with Wash Solution per wash. Invert microwells between each wash to empty fluid. Use a snapping motion of the wrist to shake the liquid from the wells. The frame must be squeezed at the center on the top and bottom to retain microwell modules during washing. Do not allow wells to dry out between steps.
8. Add 100µL IgG or IgM anti-human HRP-conjugated antibody solution to the wells.
9. Incubate for 30 minutes at room 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 100 µL One-Component Substrate to each well and incubate for 10 minutes at room temperature (18-30°C). Blue color will develop in wells with positive samples.
12. Add 100 µL Stopping Solution (1% methanesulfonic acid) to each well to stop the enzyme reaction. Blue substrate will turn yellow and colorless substrate will remain colorless. Blank or zero the plate reader against air. Read the OD of each well at 450 nm (650 nm reference, if available). The OD values should be measured within 5 minutes after the addition of Stopping Solution.

Results

1. Calculate the mean O.D. values for the duplicates of the Reference, Reagent Blank, Controls and samples.
2. Ensure that all quality control parameters have been met (see Quality Control) before reporting test results.
3. A new reference should be prepared with every test run.

QUALITY CONTROL

1. The mean O.D. of the reagent blank (zero point) 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 the duplicates of the controls or samples should be within 25% CV of the mean O.D. value for samples with absorbance readings greater than 0.250.
3. 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 (or IgM) anti-EBOV antibody levels obtained with this assay are not for use in diagnostic procedures.

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

Testing Ebola 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 test have not been established.

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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