ReEBOV® Antigen ELISA Test Kit
(EBOV Recombinant Antigen Detection)

For Research Use Only

Not for use in diagnostic procedures.

The performance characteristics of this product have not been established.

PRINCIPLE OF THE TEST

The test is performed as a direct ELISA to detect Ebola Virus (EBOV) viral matrix protein 40 (VP40) and nucleoprotein (NP). Diluted samples, reference, and controls are incubated in microwells coated with purified EBOV antigen specific polyclonal antibodies. Incubation allows the antigen present in the samples to react with the immobilized antibodies. After the removal of unbound serum or plasma proteins by washing, purified EBOV antigen specific polyclonal antibody conjugated to horseradish peroxidase (HRP) is added to complex with the bound antigen. Following another washing step, the bound anti-EBOV HRP conjugate is assayed by the addition of tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) chromogenic substrate. The intensity of color development in the wells is proportional to the serum or plasma concentration of EBOV antigen.

Results are obtained by reading the OD (optical density or absorbance) of each well in a spectrophotometer. Antigen concentration can be estimated from a serial dilution curve prepared from the Reference provided in the kit. Positive and Normal serum controls are provided. It is recommended that the user establish a cut-off for the study population using at least 100 normal (non-febrile) serum samples.

REAGENTS

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

Component	1-plate kit	10-plate kit
Anti-VP40 Coated Microwell Plate (resealable bag with desiccant)	One 12x8 plate	Ten 12x8 plate
Sample Diluent 1 (Yellow)	2 bottles (60 mL)	4 bottles (120 mL)
Antigen Reference (recombinant VP40 diluted in human plasma), lyophilized	2 vials (0.25 mL)	20 vials (0.25 mL)
Negative Control (human plasma), Lyophilized	2 vials (0.25 mL)	20 vials (0.25 mL)
Anti-VP40 HRP Conjugate Solution (Orange)	1 bottle (15 mL)	1 bottle (120 mL)
ELISA Substrate (TMB and H_2O_2); ready to use (Amber Bottle)	1 bottle (15 mL)	1 bottle (120 mL)
ELISA Stopping Solution (1% methanesulfonic acid) (Red Cap)	1 bottle (15 mL)	1 bottle (120 mL)
ELISA Wash Concentrate (33X PBS-Tween)	1 bottle (60 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 Reference and Controls included in this kit have been tested and shown to be negative for antibodies to HBsAg, HCV, and HIV 1 & 2 by FDA required tests. However, all human blood derivatives, including patient samples, should be handled as potentially infectious material.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
4. Certain components of this product may contain Sodium Azide as a preservative. Sodium Azide has been reported to form lead and copper azides when left in contact with these metals. These metal azides are explosive. Any solutions containing Sodium Azide must be thoroughly flushed with copious amounts of water to prevent the build-up of explosive metal azides in the plumbing system.
5. Wear disposable gloves while handling kit reagents and wash hands thoroughly afterwards.
6. The 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.
7. 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, EDTA plasma, or citrated plasma (3.2%) are the preferred sample matrices. Blood should be collected by venipuncture, allowed to clot, and the serum separated from the cells by centrifugation. If not tested immediately, specimens should be stored at 2–8°C. If specimens are to be stored for more than 1 week, freeze at -20°C or below. Avoid repeated freezing and thawing. Do not use 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 EDTA or citrated plasma 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® Antigen Detection ELISA Test Kit; see “Reagents” for a complete listing.

Materials Required but not Provided:
- Reagent grade water to prepare Wash Solution (2 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 for manual plate washing or an automated or semi-automated 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. Careful controlled timing of all steps is critical. All Reference, 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.
8. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
9. Incubation temperatures above or below normal room temperature (18-30°C) when required may contribute to inaccurate results.
10. Avoid contamination of reagents when opening and removing aliquots from the primary vials.
11. Do not use kit components beyond expiration date.
12. Do not use kit 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: On day of use, reconstitute Reference and Normal 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 colder) for longer periods.

Assay Procedure

1. Remove any microwell strips that will not be used from the frame. Store them with the desiccant pouch in the resealable bag provided.
2. Prepare a five-point Reference curve: Label five tubes for Reference 1 – 5.

   In tube #1, prepare a 1:10 dilution of Reference in Sample Diluent (yellow) by adding 100 µL reference to 900 µL Sample Diluent.

   Add 500 uL of Sample Diluent (yellow) 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	-	100 uL Reference	+	900 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 should be run in duplicate on each plate. These wells will be treated the same as sample wells in subsequent assay steps.
4. Duplicate determinations are recommended. Prepare a 1:10 dilution of the controls and samples in Sample Diluent (yellow), e.g., 50 µL sample added to 450 µL Sample Diluent equals a 1:10 sample dilution.
5. Mix thoroughly and add 100 µL of each sample (Reference dilutions, controls, samples, and reagent blank) to the appropriate microwells.
6. Incubate 60 minutes at 35 - 37°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 100 µL HRP Conjugated Antibody Solution (orange) to each well.
9. Incubate for 30 minutes at room temperature (18-30°C).
10. Wash 4 times as in step 7.
11. Add 100 µL Substrate (TMB, H2O2) 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. Air blank or zero the plate reader. Read the O.D. of each well at 450 nm (and 650 nm reference if dual beam). The O.D. 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 dilutions, Reagent Blank, Controls and samples.
2. Plot the mean O.D. obtained for each Reference (y axis) against the corresponding Reference value (x axis) using a Log-Log or Point-to-Point curve fit calculation.
3. Ensure that all quality control parameters have been met (see Quality Control) before reporting test results.
4. A new calibration curve 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 patient 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

Estimated EBOV antigen levels obtained with this assay are not for use in diagnostic procedures.

ReEBOV® Antigen Detection ELISA Test Kit is designed to detect circulating EBOV antigen titers during viremia stage of illness. Ebola patient samples that have progressed to a humoral immune response stage may not have detectable EBOV antigen titers. These samples may still be positive by EBOV PCR or by Ebola virus specific antibody detection assays such as ReEBOV® IgG/IgM Detection ELISA Test Kit.

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

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

Warranty

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