EBOV IgG Capture EIA

An Enzyme Immunoassay for the detection of human IgG antibodies to Ebola virus in oral fluid, serum and plasma samples

For research use only not to be used for diagnostic purposes

Cat. No: E15-060

FOR RESEARCH USE ONLY

Manufactured & Distributed by

Kalon Biological Limited
Unit G Perram Works
Merrow Lane
GUILDFORD
GU4 7BN
UK

Phone: +44 (0)1483 302 007

Fax: +44 (0)1483 301 907

Email: info@kalonbio.co.uk

INTENDED USE AND APPLICATION

An enzyme Immunoassay (EIA) for the detection of human IgG antibodies to Ebola virus in serum, plasma and oral fluid. This product is offered to trained laboratory personnel for research use only. This product is not to be used for diagnostic purposes.

SUMMARY AND EXPLANATION

The EBOV IgG Capture EIA has been developed so that glycoprotein (GP)-specific IgG antibodies can be monitored in population studies. The assay might also be useful to confirm the presence of high levels of specific antibodies in potential donors.

TEST PRINCIPLE

In the EBOV IgG Capture EIA, oral fluid extract, diluted serum or diluted plasma is added to an anti-human IgG coated microtitre well. IgG in the specimen binds to the well and after washing, EBOV Tracer consisting of enzyme-conjugated recombinant EBOV glycoprotein antigen is added. Ebola specific IgG in the sample, if present, binds the Tracer. After washing, TMB Substrate is added to initiate an enzyme reaction with a coloured end-point. The presence of EBOV-specific IgG results in a blue coloured product which becomes yellow on adding the acid Stop Solution. The yellow-coloured solution is measured using a photometric plate reader at 450 nm with background correction set between 620 and 650 nm. The presence of EBOV specific IgG is indicated by optical density values above the cut-off.

WARNINGS AND PRECAUTIONS

- Although the serum used to prepare the positive and negative controls were not reactive for antibodies to HIV 1 and 2, HCV or Hepatitis B surface antigen, the control sera should be handled and disposed of as though potentially infectious.
- TMB Substrate contains 3,3',5,5'-tetramethylbenzidine and has been reported to be non-carcinogenic. Contact with skin and mucous membranes should be avoided. Wear protective gloves when dispensing and using this reagent. If TMB Substrate comes into contact with skin and mucous membranes, rinse with copious amounts of water.
- Stop Solution contains 0.5 mol/L hydrochloric acid. Contact with skin and mucous membranes should be avoided. If Stop Solution comes into contact with these sites, rinse with copious amounts of water.
- Wear disposable gloves when handling clinical specimens and kit components. Treat all clinical specimens and controls and any materials coming into contact with them as potentially infectious.
- Dispose of clinical material and potentially infected materials in accordance with local regulations.
- Do not mix components of one lot of kits with components from other lots.
- Avoid microbial contamination of reagents. Do not use reagents that show signs of contamination.
- Good laboratory procedure should be employed to avoid cross contamination of samples and reagents. Take out only the required volume of reagent from the original container

(usually 0.9 - 1.0 mL per strip) for dispensing into wells. Discard any unused reagent - do not return to the container!

MATERIALS PROVIDED

Each kit contains one 96 well microplate and has sufficient materials to run up to 92 tests. The kit is stable up to the expiration date printed on the kit label if stored at 2-8°C.

1. ANTI-HUMAN IgG PLATE: PN 2109J, 8 × 12 microwell strips coated with anti-human IgG antibody packed in a re-sealable pouch with desiccant. Open the pouch by cutting along the notched edge and separating the re-sealable joint. Return unused strips to the pouch with desiccant and store at 2-8°C. Strips should be used within 3 months of initial opening.
2. SERUM DILUENT PN 2040, 100 mL: one bottle containing phosphate buffered saline, protein stabiliser, detergent and red dye.
3. WASH FLUID 20× PN 6F83, 125 mL: one bottle containing 20× glycine/borate buffer, detergent and preservative. Dilute 1 in 20 with purified water.
4. POSITIVE CONTROL PN E15-021, 1.4 mL: one vial containing pre-diluted convalescent serum positive for EBOV IgG antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
5. CUT-OFF CALIBRATOR PN E15-031, 1.9 mL: one vial containing pre-diluted convalescent serum positive for EBOV IgG antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
6. NEGATIVE CONTROL PN E15-041, 1.9 mL: one vial containing pre-diluted serum negative for EBOV IgG antibody in phosphate buffered saline containing detergent, protein stabiliser and antimicrobial agent.
7. EBOV TRACER PN E15-051, 12 mL: one bottle containing peroxidase-conjugated recombinant EBOV glycoprotein antigen in a buffered solution containing protein stabilisers, detergent, antimicrobial agent and red dye.
8. TMB SUBSTRATE PN R7-01, 13 mL: one bottle containing 3,3',5,5'-tetramethyl-benzidine, a peroxide source and stabilisers.
9. STOP SOLUTION PN 2031, 14 mL: one bottle containing 0.5M hydrochloric acid.
10. PLASTIC PLATE BAGS. Easy-sealing bags for incubating plate during assay.

MATERIALS REQUIRED BUT NOT PROVIDED

- Oral Fluid collection device e.g. Oracol or other similar swab (see Specimen Collection).
- Buffer for extracting oral fluid from an oral fluid collection device.
- Laboratory grade deionised or distilled water.
- Tubes suitable for diluting serum specimens and microtitre plate sealer.
- Micropipettes and disposable tips capable of delivering 1000 µL, 100 µL, 10 µL and 5 µL volumes.
- Waste discard container with disinfectant.
- ELISA plate reader capable of reading optical densities at 450 nm and 635 ± 15 nm.

- Incubator set to 37 ± 2°C.

SPECIMEN COLLECTION

Handle all oral fluid, blood, serum and plasma as potentially infectious material.

Oral fluid specimens should be collected as described on the outer package of the Oracol collection device. Other oral fluid collection devices should be validated in the assay before use.

Oral fluids should be eluted into transport medium, a buffer of neutral pH containing between 3-10% (v/v) newborn calf serum, antibacterial and antifungal reagents and optionally 0.05% Tween 20. Transport medium prepared for parallel testing in viral culture or PCR may not contain Tween 20. In this case Tween 20 should be added to a final concentration of 0.05 – 0.1%. The procedure for processing Oracol swabs used to collect oral fluid has been described in a video [(1)].

Serum and plasma (EDTA, citrated or heparinised) samples are suitable specimens for the test and should be obtained using standard procedure.

REAGENT AND SAMPLE PREPARATION

Bring all reagents to room temperature (18-25°C) prior to use.

Prepare working strength Wash Fluid by adding 1 part Wash Fluid 20× to 19 parts distilled or deionised water. It is recommended that working strength buffer be prepared as required on the day of use. Remaining Wash Fluid 20× should be stored at 2-8°C. Enough has been provided to enable 3 × 4 washes of each well.

All other reagents are provided ready to use.

Dilute serum and plasma samples 1/201 in Serum Diluent e.g. dispense 5 µL of specimen into a labelled tube and add 1 mL of Serum Diluent.

Oral Fluid samples extracted into transport medium should not be diluted further.

ENZYME IMMUNOASSAY PROCEDURE

1. Remove and assemble the required number of microwell strips to perform the test. A minimum of 4 wells is needed for the controls which must be included in each test run. Return unused microwell strips and the desiccant to the foil pouch and reseal.
2. Pipette 100 µL of the Positive Control, Cut-Off Calibrator and Negative Control to each assigned well: one well for the Positive Control, two wells for the Cut-Off, and one well for the Negative Control.

   Pipette 100 µL of the undiluted oral fluid extract or the diluted serum specimens to assigned wells. Only test the number of samples in a single test run that can be dispensed within five minutes. *Note: this step can be accomplished more quickly if controls and test samples pre-dispensed into microplate compatible tubes or a microtitre holding plate then transferred to the test plate using a multichannel pipette.*

   Seal in the plastic bag provided and incubate at 37 ± 2°C for 60 ± 2 minutes.
3. Wash wells four times with working strength Wash Fluid (see reagent preparation). The wash cycle is carried out as follows: aspirate the contents of the well and dispense

350 µL/well of diluted Wash Fluid, leave to soak for approximately 30 seconds and aspirate. Repeat the wash cycle three further times. It is recommended to use an automatic plate washer for this procedure. Tap the wells dry on absorbent paper.

4. Pipette 100 µL of EBOV Tracer to each well, seal in the plastic bag provided and incubate at 37 ± 2°C for 120 ± 4 minutes.
5. Wash wells four times with working strength Wash Fluid as in step 3.
6. Pipette 100 µL of TMB Substrate to each well. This is best performed with a multichannel pipette. Incubate for 30 ± 1 minutes, protected from strong light at room temperature (18-25°C).
7. Pipette 100 µL of Stop Solution to each well. This reagent should be added to wells in the same order as step 12 so that the timing is accurate.
8. Read the optical densities (OD) at 450 nm in an ELISA plate reader. If the feature is available, set the reference wavelength between 620 and 650 nm.

QUALITY CONTROL.

The assay can be considered valid if the protocol has been followed correctly, the Positive Control optical density (OD) is greater than 0.8 and the ratio of the Cut-off Calibrator OD to the Negative Control OD is greater than 2.0.

INTERPRETATION OF RESULTS

Calculate the mean OD of the two Cut-off Calibrator wells, this is the Cut-off value ($\overline{CO}$). Calculate the Antibody Index (AI) of each specimen by dividing the specimen OD by $\overline{CO}$.

Score results with an Antibody Index greater than 1.1 as reactive.

Score results with an Antibody Index less than 0.9 as non-reactive. A non-reactive result indicates that EBOV IgG was not detected in the sample. (see limitations of the test).

Test results falling within 10% of the Antibody Index are equivocal and should be repeated to confirm their status: -

EBOV IgG Reactive

Specimen AI > 1.1

EBOV IgG Non-Reactive

Specimen AI < 0.9

Equivocal result

0.9 ≤ Specimen AI ≤ 1.1

LIMITATIONS OF THE TEST

Microbiological contamination of the specimens may lead to erroneous results.

Oral fluid samples with low total immunoglobulin concentration (less than 1 µg/mL) are not suitable for use in this test and may give rise to erroneous results.

The EBOV IgG Capture EIA detects antibodies specifically to EBOV glycoprotein antigen. Antibodies to other virus proteins are not detected in this assay.

The patient's profile, epidemiological data and the test results should all be considered when considering the results of this assay.

TEST PERFORMANCE

The results obtained in the Kalon EBOV Capture EIA with convalescent donor serum (n = 16) and oral fluid (n = 28) were in complete agreement with the reference assay [2,3]. See tables 1 and 2.

In addition, 30 serum samples from South Africa and 49 samples from Kisumu, Kenya were assayed in the Kalon EIA. These are regions where sero-positivity for Ebola virus is not to be expected (but where, in the case of Kisumu, malaria is prevalent). The assay results for all samples were non-reactive from both groups. South Africa mean AI = 0.30 (range 0.13 – 0.71); Kenya mean AI = 0.23 (range 0.14 – 0.57).

Table 1. Results obtained with convalescent donor serum in the Kalon EIA compared to the reference assay.

Reference Assay	Kalon EBOV IgG Capture EIA			
	Non-reactive	EQV	Reactive	TOTAL
Non-reactive	2			2
EQV				
Reactive			26*	26
TOTAL	2		26	28

* mean AI = 5.8 (range 3.2 – 9.8)

Table 2. Results obtained with oral fluid from convalescent donors in the Kalon EIA compared to the reference assay.

Reference Assay	Kalon EBOV IgG Capture EIA			
	Non-reactive	EQV	Reactive	TOTAL
Non-reactive	1			1
EQV				
Reactive			15*	15
TOTAL	1		15	16

* mean AI = 4.4 (range 1.4 – 7.6)

REFERENCES

1. Medical training video: oral fluid samples. Public Health England (2013). Accessed 04-Dec-2015 at *https://www.youtube.com/watch?v=6wDDLp_OaT*c.
2. Glynn JR, Bower H, Johnson S, Houlihan CF, Montesano C, Scott JT, Semple MG, Bangura MS, Kamara AJ, Kamara O, Mansaray SH, Sesay D, Turay C, Dicks S,

Wadoum REG, Colizzi V, Checchi F, Samuel D, Tedder RS. Asymptomatic infection and unrecognised Ebola virus disease in Ebola-affected households in Sierra Leone: a cross-sectional study using a new non-invasive assay for antibodies to Ebola virus. Lancet Infect Dis. 2017 Jun;17(6):645-653.

3. Baker S, Dicks S, Ijaz S, Kargbo O, Niazi S, Nkwanma D, Philip , Scott J, Semple C, Tedder RS, Umberto A. Serological support for the public health intervention of convalescent plasma in Ebola disease. 2016 Draft for publication.

WARRANTY

The product is warranted to perform as described in the labelling and in the product insert when used as instructed. NO WARRANTY EXTENDS BEYOND THIS. KALON BIOLOGICAL LTD DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. Kalon Biological's sole obligation and the purchaser's exclusive remedy for breach of this warranty shall be at the option of Kalon Biological Ltd to replace the products and in no event shall be liable for any proximate, incidental or consequential damage in connection with this product.

SUMMARY OF ASSAY PROTOCOL

Bring All Reagents to Room Temperature

Dilute Wash Fluid 20× in water (1 + 19) as required.

Dilute Test Serum or Plasma in Serum Diluent (1 + 200)

	Volume per well	**Incubation Time and Temperatures**
1. Assemble required number of coated strips into plate frame		
2. Pipette Controls and Cut-Off, 1 × PC, 2 × CO, 1 × NC, and all oral fluid extract, diluted serum and plasma test specimens. Complete this step within 5 minutes.	100 µL	60 ± 2 min at 37 ± 2°C
3. Wash with working strength Wash Fluid	4 × 350 µL	
4. Pipette Tracer	100 µL	120 ± 2 min at 37 ± 2°C
5. Wash with working strength Wash Fluid	4 × 350 µL	
6. Pipette TMB Substrate	100 µL	30 ± 1 min at Room Temperature (18 to 25°C) in the dark.
7. Pipette Stop Solution	100 µL	
8. Read Optical Density @ 450 nm with reference set to 635 ± 15 nm		

Ref: K50p201703