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User's Manual

Filaria IgG4 ELISA Kit

REF **DEIA1104S**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Filaria IgG4 ELISA is for qualitative detection of specific IgG4 antibodies in specimens to highly specific antigen IWb123 target antigen expressed primarily in infective stage larvae (L3) of the lymphaticdwelling parasite Wuchereria bancrofti (Wb). This product is for research use only. Not for use in diagnostic procedures.

Principles of Testing

The Filaria IgG4 ELISA consists of one enzymatically amplified sandwich-type immunoassay. Positive, negative and low positive control samples are provided to maintain the kit's integrity. In this assay, control samples and unknown serum samples are diluted

into the Filaria Sample Dilution Buffer, then incubated in microtiter wells which have been coated with IWb123 (1- 6), followed by incubation with anti-human IgG4 antibody labeled with the enzyme horseradish peroxidase (HRP). After the incubation and a washing step, the wells are incubated with a tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. The absorbance measured is directly proportional to the concentration of specific IgG4 antibodies to IWb123 present. A set of positive and negative controls are provided as internal controls. These are provided to monitor the integrity of the kit components.

Reagents And Materials Provided

The Filaria IgG4 ELISA kit contains sufficient reagents for one plate of 96 wells (8 x12 strips) each. The kit contains the following reagents:

Warning: Do not use any reagents where damage to the packaging has occurred.

1. FILARIA ANTIGEN COATED MICROTITER STRIPS
2. FILARIA SAMPLE DILUTION BUFFER:
3. FILARIA NEGATIVE CONTROL
4. FILARIA LOW POSITIVE CONTRO
5. FILARIA POSITIVE CONTROL
6. 100X ENZYME CONJUGATE FOR FILARIA
7. CONJUGATE DILUENT FOR FILARIA
8. 10X WASH BUFFER
9. LIQUID TMB SUBSTRATE
10. STOP SOLUTION

Warning: strong acid, wear protective gloves, mask and safety glasses. Dispose all materials according to safety rules and regulations.

Materials Required But Not Supplied

1. ELISA pectrophotometer capable of absorbance measurement at 450 nm. (Do not use background subtraction or other wavelengths)
2. Biological or High-Grade Water
3. Vacuum Pump
4. Manual or Automatic Plate Washer or **Wash Bottle
5. *37 °C Incubator without CO2 supply (It is highly recommended that a secondary source other than the incubator visual display be used to measure the temperature prior to kit use.
6. 1-10 µL Single-Channel Pipettors, 50-200 µL Single-and Multi-Channel Pipettors
7. Polypropylene tubes
8. Parafilm or plate sealers
9. Timer
10. Vortex

*Improper adherence to these can critically affect kit performance and results.

** In the instance were a water bottle is used, care must be taken to (1) not touch the well surface with the bottle tip, (2) to add the equivalent of 300 µl to each well for each wash cycle, (3) to avoid run over from one well into another, (4) to empty each well completely between washes. This is best achieved by inverting the plate and gently tapping on an absorbent surface.

Storage

Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles.

Specimen Collection And Preparation

Only serum or dried blood spots should be used for this assay, and the usual precautions for venipuncture should be observed. Blood obtained by venipuncture should be allowed to clot at room temperature (20-25 °C) for 30 to 60 minutes and then centrifuged according to the Clinical and Laboratory Standards Institute (CLSI Approved Guideline – Procedures for the Handling and Processing of Blood Specimens; Second Edition. H18-A2 ISBN 1-56238-388-4).

Hyperlipemic, heat-inactivated, hemolyzed, or contaminated sera may cause erroneous results; therefore, their use should be avoided.

Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods. Separated serum should remain at 20-25C for no longer than 8 hours. If assays are not completed within 8 hours,

serum should be refrigerated at 2-8 °C. If assays are not completed within 48 hours, or the separated serum is to be stored beyond 48 hours, serum should be frozen at or below -20 °C.

Avoid repeated freezing and thawing of samples since this can cause analyte deterioration. Frostfree freezers are not suitable for sample storage.

Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.

If sera are to be shipped, they should be packed in compliance with Federal Regulations covering

transportation of infectious agents.

Do not use sera if any indication of microbial growth is observed.

Reagent Preparation

1. Preparation of 1X Wash Buffer:

Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 120 ml 10X wash buffer with 1080 ml distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved. After diluting to 1X, store at

room temperature for up to 6 months. Check for contamination prior to use. Discard if contamination is suspected.

2. Preparation of working solution of Enzyme Conjugate HRP: Dilute 100X Enzyme Conjugate for Filaria in the

Conjugate Diluent provided. For one plate, prepare 12ml of diluted conjugate by adding 1 part 100x enzyme conjugate to 100 parts conjugate diluent. For example, make a 100X diluted conjugate by adding 120ul of 100X enzyme conjugate to 12ml of conjugate diluent. Mix well by end-to-end slow mixing. This solution may be stored for up to 2 weeks if stored at 2-8ºC. After 2 weeks, this conjugate solution should be discarded and no longer used in this assay.

3. Microtiter Strip Wells: Select the number of coated wells required for the assay. The remaining unused wells should be placed back into the pouch quickly, sealed, and stored at 2-8 °C until ready to use or expiration.

Assay Procedure

1. Positive, negative and low positive controls should be assayed in duplicate. Unknown serum samples to be tested can be assayed singlet or in duplicate. Refer to the flow chart at the end of this section for an example of this procedure. Up to ninety test specimens can be tested in singlet, on one 96 well plate.
2. Mark the microtiter strips to be used.
3. Dilute test seraa and controls to 1/50 using the Filaria Sample Dilution Buffer. Use small polypropylene tubes for these dilutions and at least 4 uL of sera and 5 uL the provided kit controls. For example: mix 4uL of test sample plus 196 uL of the Filaria Sample Dilution Buffer SDB. For kit controls, it is recommended to mix 5 uL of control plus 245 uL of the sample dilution buffer to make a 1/50 dilution.

a. Dry Blood Spot (DBS) application: In case of DBS use, please refer to the quick

guidance procedure (Box 2).

4. Apply 100 uL per well of 1/50 diluted test sera, Negative Control, Positive Control, and Low Positive Control to the plate by single or multi-channel pipette as appropriate.

Note: For runs with more than 32 wells it is recommended that each diluted sample first be added to a clean microtiter dilution plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Filaria Antigen Coated Wells with a 100 µL 8- or 12- channel pipettor, or using a validated liquid automation system.

A suggested arrangement for 96 samples, including controls, is shown in "Example for Serum Sample Application" chart at the end of this package insert.

5. Cover the plate with parafilm just on the well opening surface, so the bottom of the plates is not covered.
6. Incubate the plate at 37 °C for 30±2 minute in an incubator.

Note: Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use CO2 or other gas incubators. Do not place plates in contact with any wet substances such as wet paper towels, etc.

7. After the incubation, wash the plate 6 times with an automatic plate washer using 1x wash buffer. Use 300 ul per well in each wash cycle.
8. Add 100 ul per well of 1:100 diluted Enzyme Conjugate HRP into all wells by multi-channel pipette.
9. Cover the plate with parafilm just on the well opening surface. The bottom of the plate should not be covered (see step 5).
10. Incubate the plate at 37 °C for 30 ± 2 minute in an incubator (see step 6).
11. After the incubation, wash the plate 6 times with an automatic plate washer using 1x wash buffer. Use 300 ul per well in each wash cycle.
12. Add 100 ul per well of Liquid TMB substrate into all wells by multi-channel pipettor.
13. Incubate the plate at room temperature (20-25 °C) in a dark place (or container) for 10 ± 1 minutes without any cover on the plate.
14. After the incubation, add 50 ul per well of Stop solution into all wells by using a multi-channel pipettor and incubate at room temperature for 1 minute without covering the plate. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, the color should change from blue to yellow.
15. Gently blot the outside bottom of wells with a lintfree paper towel (KimWipe) to remove droplets that may interfere with reading by the spectrophotometer.

Do not rub with the paper towel as it may scratch the optical surface of the well. (Note: Large bubbles on the surface of the liquid may affect the OD readings.)

16. After the incubation, read the RAW OD 450 nm (optical density at 450 nm) value with a Microplate reader. Do NOT subtract background OD values or use a reference wavelength. Analyze the results as described in the Quality Control and Interpretation of Results sections.

Calculation

Determination of the cut-off using specimens from endemic locations MUST be performed as described below.

Calculation of Cut-off value: No fixed cut-off value is provided as the cut-off will vary depending onon the disease prevalence in the geographical location where the kit is being used. Therefore, it is required that the end users MUST calculate cut-off values first using geographically relevant specimens. A inimum of 100 specimens from each of 3 categories - diseased (confirmed with filaria), confirmed

unrelated febrile and related parasitic diseases (e.g. Loa loa, Onchocerca volvulus and M. streptocerca streptocerc, and other potentially cross-reactive diseases prevalent in a given area including malaria, typhoid fever, etc.), and normal healthy adults from endemic areas – are recommended for determination of the appropriate cutoff. Receiver Operating Characteristic (ROC) curves can be used to determine a cut-off value.

Note: Once a cut-off value is determined from a given

location, the value can be used for future reference and calculation. The fixed cut-off must be verified or validated for consistency using a set of in-house panel samples available to the end users. They are to be used in combination with controls provided with the kits.

Ensuring Assay Validity: The results on the table below must be obtained using provided positive and negative controls to calculate discrimination capacity of the assay. Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Kit controls (positive, low positive and negative control) are provided to assess ONLY the kit integrity. The Discrimination Capacity is defined as the ratio of the mean Positive Control OD450 to the mean Negative Control OD450 (PC ÷ NC).

Interpretation Of Results

Interpretation of the results:

1. Samples with spectrophotometric readings greater than the geographically established Cutoff value are considered to be "Reactive" and samples below this criterion are considered to be

"Non-Reactive".

2. Any "Reactive" sample must be repeated to verify the result. Values near the Cut-off are considered to be doubtful and the assay must be

repeated in triplicate to establish the sample status.