

**AccuDiag™
 Filaria IgG4
 ELISA**

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES

Cat# 8211-25

 See external Label  2-8°C  Σ=96 Tests

Test	Filaria IgG4
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA : Antigen Coated Plate
Detection Range	Qualitative: Positive & Negative Control
Sample	10µl Serum
Total Time	~ 60 min
Shelf Life	12-14 Months

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 lymphatic-dwelling parasite *Wuchereria bancrofti* (Wb). This product is for research use only. Not for use in diagnostic procedures.

TEST PRINCIPLE

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.

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 20-25°C 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. Frost-free 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.

MATERIALS AND COMPONENTS

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:** Strip holder in Ziploc foil, containing 96 polystyrene microtiter wells coated with IWb123 in each well. Stable at 2-8°C until the expiration date.
2. **FILARIA SAMPLE DILUTION BUFFER:** Two bottles, 25 ml, Tris-HCl buffered solution (pH 7.2-7.6) with Tween 20 (0.05%), preservative (0.05% proclin-300) and additives. Use for the dilution of test samples, positive and negative controls. This sample dilution buffer is also used for the elution of dried blood spots as described in the Dry Blood Reference guide below. Stable at 2-8°C until the expiration date.
3. **FILARIA NEGATIVE CONTROL:** One vial, 50 µl. Negative serum. The Negative Control will aid in monitoring the integrity of the kit. Store at 2-8°C.
4. **FILARIA LOW POSITIVE CONTROL:** One vial, 50 µl. Low reactive positive sample. The Low Positive Control will aid in monitoring the integrity of the kit. Store at 2-8°C.
5. **FILARIA POSITIVE CONTROL:** One vial, 50 µl. Positive serum containing <1.0% BSA and <0.05% Proclin 300. The Positive Control will aid in monitoring the integrity of the kit. Store at 2-8°C.
6. **100X ENZYME CONJUGATE FOR FILARIA:** One vial, 150ul, contains Mouse monoclonal anti-human IgG4 conjugated with horseradish peroxidase (HRP) in Tris

buffered solution (pH~7.4), preservative and additives.
 Store at 2-8°C until the expiration date.

7. CONJUGATE DILUENT FOR FILARIA: One bottle, 14ml contains PBS buffered solution (pH~7.4) with preservative and additives. The 100X Enzyme Conjugate is diluted into this Conjugate Diluent before use. Stable at 2-8°C until expiration date.
8. 10X WASH BUFFER: One bottle, 120 ml, of 10X concentrate of phosphate buffered saline with Tween 20 (pH 6.8-7.0). Stable at 2-8°C until the expiration date.
9. LIQUID TMB SUBSTRATE: One bottle, 12ml, ready to use. Contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide in a citric-acid citrate buffer (pH 3.3-3.8). Stable at 2-8°C until the expiration date.

Note: The TMB substrate should always be stored in the light-protected bottle provided.

10. STOP SOLUTION: One bottle, 6 ml, of ready to use 1N Sulfuric Acid. Used to stop the enzymatic turnover of the substrate. Stable at 2-8°C until the expiration date.

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

Materials required but not provided

- *ELISA Spectrophotometer capable of absorbance measurement at 450 nm. (Do not use background subtraction or other wavelengths)
- Biological or High-Grade Water
- Vacuum Pump
- Manual or Automatic Plate Washer or **Wash Bottle
- *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.
- 1-10 µL Single-Channel Pipettors, 50-200 µL Single-and Multi-Channel Pipettors
- Polypropylene tubes
- Parafilm or plate sealers
- Timer
- Vortex

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

**** In the instance where 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 300µl 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.**

TEST PROCEDURE

CAUTION: The test procedure must be strictly followed. Any deviations from the procedure may produce erroneous results. Bring all kit reagents and specimens to room temperature (~25°C) before use. Thoroughly mix the reagents and samples before use by gentle inversion. NOTE: For long-term storage, serum samples should not be repeatedly thawed and frozen more than three times. Sera should be further aliquoted in a smaller volume and stored at -20C.

PREPARATION OF REAGENTS

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.

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 12mls of conjugate diluent. Mix well by end-to-end slow mixing. *This solution may be stored for up to 2 weeks if stored at 2-8°C. After 2 weeks, this conjugate solution should be discarded and no longer used in this assay.*

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 sera and controls to 1/50 using the Filaria Sample Dilution Buffer. Use small polypropylene

tubes for these dilutions and at least 4 µL of sera and 5 µL the provided kit controls. For example: mix 4 µL of test sample plus 196 µL of the Filaria Sample Dilution Buffer SDB. For kit controls, it is recommended to mix 5 µL of control plus 245 µL 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).

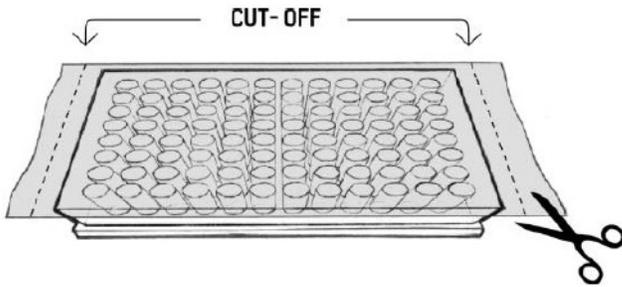
4. Apply 100 µL per well of 1/50 diluted test sera, Negative Control, Positive Control, and Low Positive Control to the plate by single or multi-channel pipette as appropriate.

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.

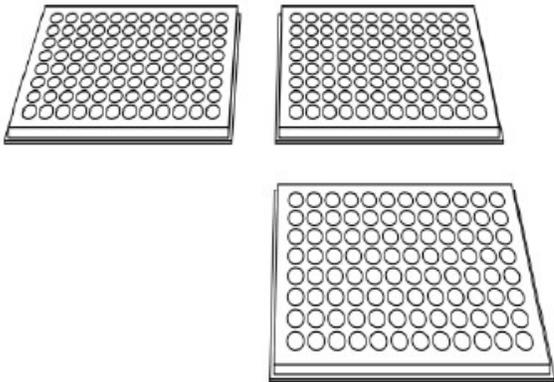
A suggested arrangement for 96 samples, including controls, is shown in "Example for Serum Sample Application" chart at the end of this package insert.

5. Cover the plate with parafilm just on the well opening surface, so the bottom of the plates is not covered.

Note: This is to ensure even temperature distribution in all wells from bottom and sides; any extra parafilm can be cut off once the top is sealed to block evaporation.



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.



CORRECT METHOD

7. After the incubation, wash the plate 6 times with an automatic plate washer using 1x wash buffer. Use 300 µl per well in each wash cycle.
8. Add 100 µl 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 µl per well in each wash cycle.

12. Add 100 µl 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 50µl 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 lint-free 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.

Serum Use Quick Reference Guide

1. Dilute serum samples and Controls: 1/50 in Sample Diluent. **Controls should be run in duplicate.**
2. Add 100 µl of sample to the wells. Cover and incubate for 30 minutes at 37 °C.
3. Wash 6 times.
4. Add 100 µl of 1:100 diluted Enzyme Conjugate HRP to each well. Cover and incubate for 30 minutes at 37 °C.
5. Wash 6 times.
6. Add 100 µl of TMB solution and incubate for 10 minutes, reducing exposure to light.
7. Add 50 µl of Stop Solution.
8. Incubate the plate at room temperature for 1 minute.
9. Measure the optical density at λ = 450 nm. Do Not subtract any background absorbance values.

Please see the PROCEDURE section for important details.

Dry Blood Spot (DBS) Use Quick Reference Guide

1. Apply 10 µl of whole blood (WB) samples per spot on the filter paper.
2. Filter spots should be dried at RT for 18-24 hrs. (or “overnight dry”).
3. Cut off the dried spot from main body of the filter paper.
4. Place the dried blood spot in a suitable tube; add 500 µl of the Filaria Sample Dilution Buffer into the tube containing the filter spot.
5. Place the tube in any appreciate tube rack rotator and “roll” the tube continuously.
6. Continue extraction at 4 °C for 18-24 hrs. (or “overnight elution”).
7. 100 µl of the extracted sample should be used directly in the Filaria IgG4 ELISA in place of the 1:50 diluted sample.
8. Controls samples must be diluted 1:50 in the Sample Dilution buffer and included in the assay.

The rest of the test procedure is same as described in Box 1 for serum.

Please see the PROCEDURE section for important details.

RESULTS

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 on 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 minimum 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 cut-off. 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/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).

Quality Control Requirements

Factor	Tolerance
Mean Negative OD ₄₅₀ (NC)	< 0.200
Mean Positive OD ₄₅₀ (PC)	> 0.500
Discrimination capacity (R _{PCNC})	> 5

Interpretation of the results:

1. Samples with spectrophotometric readings greater than the geographically established Cut-off 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.

LIMITATIONS OF PROCEDURE

- The kit is designed for research use only. Not for diagnostic use
- The reagents supplied in this kit are optimized to measure IWb123 antigen reactive IgG4 antibody levels in serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided. Do not freeze the liquid TMB substrate.
- Hemolyzed and lipemic specimens may give false values and should not be used.
- The assay performance characteristics have not been established for visual result determination.
- Serum and Plasma Comparisons: The assay described here has been optimized with serum. Care should be taken on the quality of sample. Particulate, lipemic, hemolysed and aged samples should not be used.

- Kits performance has not been assessed in immunocompromised population, or in population with autoimmune diseases, or any lymphoproliferative diseases.
- No interference studies have been performed in presence of common interferents, such as hemoglobin, excessive lipids, cholesterol, etc.

PRECAUTIONS

FOR RESEARCH USE ONLY - NOT FOR USE IN DIAGNOSTIC PROCEDURES.

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.

SAFETY PRECAUTIONS

- All human source materials used in the preparation of controls have been either heat inactivated or tested negative for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. However, no test method can ensure 100% efficiency. Therefore, all human controls and antigen should be handled as potentially infectious material. The Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
- Wear protective clothing, eye protection, and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke, or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipette by mouth.

TECHNICAL PRECAUTIONS

- This test must be performed on serum or dried blood spot samples. See Box 2 for dried blood spot preparation. The use of whole blood, plasma or other specimen matrix has not been validated.
- Do not mix various lots of any kit component within an individual assay.
- Do not heat inactivate test sera.**
- Cover working area with disposable absorbent paper.
- Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
- All reagents must be equilibrated to room temperature (20-25°C) before commencing the assay. The assay will be affected by temperature changes.
- Avoid repeated freezing and thawing of the serum specimens to be evaluated.
- Dispense reagents directly from bottles using clean pipette tips. Transferring reagents may result in contamination.
- Unused microwells must be resealed immediately and stored in the presence of desiccant. Failure to do so may cause erroneous results with those unused microwells.
- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Some reagents may form a slight precipitate, mix gently before use.
- Incomplete washing will adversely affect the outcome and assay performance.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents. Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP.

WARNING:

POTENTIAL BIOHAZARDOUS MATERIAL

This kit contains reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against

microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

CHEMICAL HAZARD

Safety Data Sheets (SDS) are available for all components of this kit. Review all appropriate SDS before performing this assay. Avoid all contact between hands and

eyes or mucous membranes during testing. If contact does occur, consult the applicable SDS for appropriate treatment.

REFERENCES

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6. Steel C, Kubofcik J, Ottesen EA, Nutman TB. 2012. Antibody to the filarial antigen Wb123 reflects reduced transmission and decreased exposure in children born following single mass drug administration (MDA). *PLoS Negl. Trop. Dis.* 6:e1940.10.1371/journal.pntd.0001940

<p>ISO 13485 ISO 9001</p> 			
 <p>Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa St. Suite 102 and 116, Woodland Hills, CA 91367 USA</p>			
Date Adopted	2017-08-16		
Reference No. Cat # 8211-25	AccuDiag™ - Filaria IgG4 ELISA		
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EC	REP		
Revision Date: 2015-11-17			