Creative Diagnostics®

Version 12-11/25

User's Manual

Human Anti-B. Pertussis FHA IgG ELISA Kit

REF **DEIA-XY70**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Human Anti-B. Pertussis FHA IgG ELISA Kit detects and quantifies B. pertussis FHA-specific IgG in serum or plasma of vaccinated or immunized humans. This ELISA is suitable for:

1. Determining immune status relative to non-immune controls;
2. Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;
3. Qualifying and/or standardizing vaccine batches and protocols.

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

General Description

Pertussis, also known as Whooping Cough, is a highly contagious disease caused by Bordetella pertussis bacteria. Vaccines for pertussis, available in combination with vaccines for tetanus, diphtheria, H. influenza B, hepatitis & polio, use acellular components, primarily the inactivated pertussis toxin. The toxin, a protein exotoxin, produced only by B. pertussis, is central to pertussis pathogenesis; vaccination with the toxoid elicits high levels of protection from the disease. Also included are two other highly immunogenic pertussis proteins: pertactin and filamentous hemagglutinin (FHA). It is one of two hemagglutinins produced by phase I strains of B. pertussis. FHA is a protein with ~ 200 Kda. which binds to sulfatides that are found on cilia of epithelial cells. Once anchored, the bacterium produces tracheal cytotoxin, which stops the cilia from beating. This prevents the cilia from clearing debris from the lungs, so the body responds by sending the host into a coughing fit. These coughs expel some bacteria into the air, which are free to infect other hosts.

The Anti-B. Pertussis FHA IgG ELISAs will quantify antibodies produced by vaccines as well as from infection with the toxin-producing organisms.

Principles of Testing

The Human Anti-FHA IgG ELISA kit is based on the binding of human anti-FHA IgG in samples to FHA immobilized on the microwells, and anti-FHA IgG antibody is detected by anti-human IgG specific antibody conjugated to HRP. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-FHA IgG present in the sample. Stopping Solution is added to terminate the reaction, and A450nm is then measured using an ELISA reader. The activity of human IgG antibody in samples is calculated relative to anti-FHA calibrators.

Reagents And Materials Provided

1. Wash Solution Concentrate (100×), 10ml.
2. Sample Diluent Concentrate (20×), 10ml.
3. Anti-Human IgG-HRP Conjugate Concentrate (100×), 0.15ml.
4. FHA Coated Strip Plate, 8-well strips (12). Coated with FHA, and post-coated with stabilizers.
5. Anti- FHA Calibrators, 0.65ml. Four (4) vials, each containing anti-FHA; in buffer with antimicrobial. 1 U/ml,

2.5 U/ml, 5 U/ml, 10 U/ml.

6. Human Anti-FHA IgG Positive Control. 0.65ml. Human anti-FHA; diluted in buffer with protein, detergents and antimicrobial. Net OD > 0.5.
7. Low NSB Sample Diluent. Reduces non-specific binding. 30 ml. Buffer with protein, detergents and antimicrobial. Use as is for sample dilution.
8. TMB Substrate. 12 ml. Chromogenic substrate for HRP containing TMB and peroxide.
9. Stop Solution. 12 ml. Dilute sulfuric acid.

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml.
2. Disposable glass or plastic 5-15ml tubes
3. Stock bottle to store diluted Wash Solution; 0.2 to 1L.
4. Distilled or deionized water to dilute reagent concentrates.
5. ELISA reader at 450 nm and ELISA plate washer

Storage

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label.

Specimen Collection And Preparation

1. Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

2. Antibody Stability & Sample Diltuion

Initial dilution of serum into Working Sample Diluent is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into Low NSB Sample Diluent, which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same day as the assay.

3. Assay Design

Review Interpretation of Results before proceeding:

Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the 1 U/ml Calibrator. This is usually 1:100 or greater dilution for human serum with normal levels of IgG and

IgM.

Run the Human Anti-FHA IgG Positive Control; net OD > 0.5.

Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.

Run a set of Calibrators, which validate that the assay was performed to specifications: 10 U/ml should give a high signal (>1.5 OD); 1 U/ml should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results).

Plate Preparation

Plate Set-up

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.

Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.

Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Reagent Preparation

1. Wash Solution Concentrate (100×). Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at 4°C for long term and RT for short term.

2. Sample Diluent Concentrate (20×). Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as Working Sample/Conjugate Diluent and store at 2-8°C until the kit lot expires or is used up.

3. Anti-Human IgG-HRP Conjugate Concentrate (100×). Peroxidase conjugated anti-human IgG in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample/Conjugate Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100× to 2-8°C storage.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE.

After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

Add 100ul of calibrators, samples and controls each to pre-determined wells.

Tap the plate gently to mix reagents and incubate for 60 minutes.

Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

Add 100ul of diluted Anti-Human IgG HRP to each well.

Incubate for 30 minutes.

Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

Add 100ul of Stop Solution to each well.

Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.

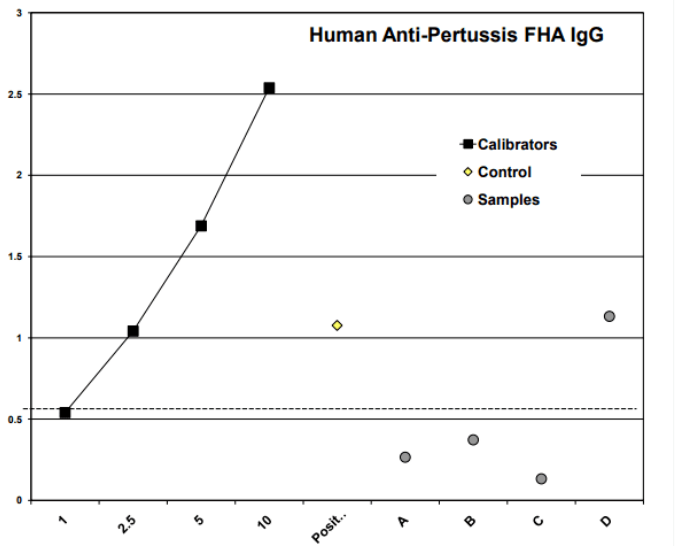
Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

Interpretation Of Results

A. Antibody Activity Threshold Index

Compare Samples to 1 U/ml Calibrator or Internal Control = Positive/Negative Cut-off.

Example:



Results:

The sensitivity of the assay to detect anti-FHA IgG, from either natural infection or vaccination, is controlled

so that the 1 U/ml Calibrator represents a threshold OD for most true positives in human serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from FHA immunization, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

1 U/ml: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between Positive/Negative. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – a human serum showing natural reactivity to FHA; net OD > 0.5. This Control can be used to normalize between-assay variation.

Samples A,B,C,D – 3 samples (1/100) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

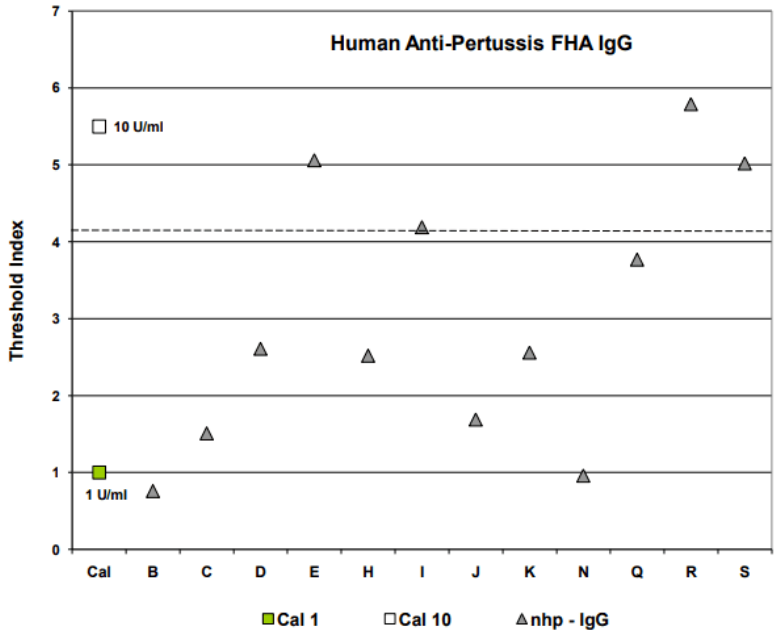
The 1 U/ml Calibrator can be used to calculate a Threshold Index that numerically discriminates Positive/Negative:

> Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

Example:

Human Serum/Plasma IgG

A panel of human serum/plasma of unknown history were tested for anti-FHA IgG (1/200 dilution). Threshold Index was calculated using the 1 U/ml Cal.



Results:

Anti-Pertussis FHA IgG: almost all NHS/P were positive at 1:200 dilution. When a significant portion of the positives are >4 Index, it may be more useful to run dilution curves to calculate titers (see next page).

Note: Positives may be expected due to prior infection and/or vaccination with B. pertussis.

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

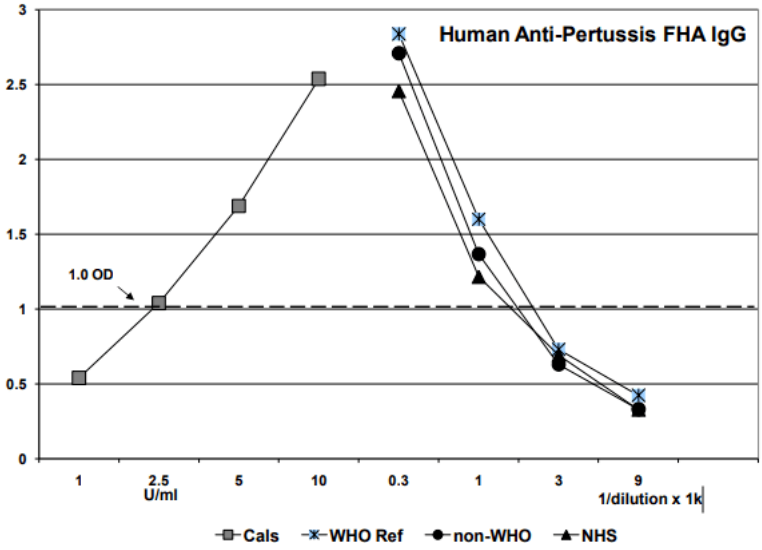
1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = Positive Index.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also quantifies the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

C. Antibody Titer

The most accurate method for comparing antibody potencies is by calculation of a titer, using an OD reading midrange in the dilution curves of each antibody as Index. In the example below, IgG titers were calculated as inverse of the dilution that produced a 1.0 OD in the assay.



Results:

Calibrators: Titer: 2.3 U/ml. The Calibrator titer value can be used to normalize between-assay sample titer values. **Note:** 1 U/ml = 24.7 mIU/ml WHO Reference

WHO Reference 1st RR: preparation 06/142, human anti-pertussis (FHA: 122 IU/ml) established as immunoassay reference.

Titer: 2.15 k (56.7 mIU FHA/ml).

Non-WHO Reference: (NIBSC 89/530) human anti-pertussis sera from infection or vaccination, established as immunoassay reference. Titer: 1.7 k

NHS: a human serum (NHS) of unknown history. Titer: 1.55 k

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit, or the WHO Std), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. In cases of non-parallelism, antibody activity is best expressed as a titer

relative to the titer of a reference positive, as shown above.

Calibrator Values

The calibrators are dilutions of antibody reactive to FHA. Values are assigned as arbitrary anti-FHA activity units.

Sensitivity

The FHA coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate anti-FHA IgG from background (non-antibody) signal with human serum samples diluted 1:100.

Specificity

Purified pertussis FHA (200 kDa) is used to coat the microwells; thus the assay is specific for antibodies directed to pertussis FHA. The anti-human IgG HRP conjugate reacts with human IgG antibodies that bind to FHA on the plate. IgA, IgM and IgE class antibodies would not be measured above background signals.

Precautions

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.