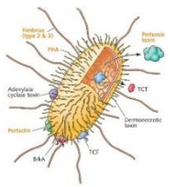
INTENDED USE

The **Human Anti-B. Pertussis ACT (Adenyl Cyclase Toxin) IgG** ELISA Kit detects and quantifies *B. pertussis* ACT-specific IgG in serum or plasma of vaccinated or immunized humans. This ELISA is suitable for:

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

This kit is for research use only, not for diagnosis or therapeutic use.

GENERAL INFORMATION



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 toxin. The **toxin (PTX)**, 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 in whole pertussis vaccines are several highly immunogenic antigens: **pertactin (PRN or p69),** an outer membrane protein ,filamentous hemagglutinins (**FHA**), antigenically distinct fimbriae proteins (**FIM2/3),** and **LPS.** Adenyl cyclase toxin (**ACT**), a virulence factor secreted by *B. pertussis*, which modulates and suppresses the host immune system contributing to pathogenesis, may also be included in several vaccines.

PRINCIPLE OF THE TEST

The Human Anti-ACT IgG ELISA kit is based on the binding of human anti-ACT IgG in samples to ACT immobilized on the microwells, and anti-ACT 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-ACT 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-ACT calibrators.

PRODUCT SPECIFICATIONS

Specificity
Purified recombinant (E. coli) adenyl cyclase toxin is used to coat the microwells; thus the assay is specific for antibodies directed to ACT.

Assay Sensitivity
The ACT coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate anti-ACT IgG from background (non-antibody) signal with human serum samples diluted 1:250.

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

KIT CONTENTS

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. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	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.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	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.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.2a11, 0.15ml	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 100X to 2-8° C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Pertussis ACT Coated Strip Plate	960-511	8-well strips (12)	Coated with ACT, and post-coated with stabilizers.
Anti- Pertussis ACT Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	960512B 960512C 960512D 960512E	0.65ml 0.65ml 0.65ml 0.65ml	Four (4) vials, each containing anti-ACT; in buffer with antimicrobial.
Anti-Pertussis ACT Positive Control	960-512-PC	0.65ml	Anti-ACT; diluted in buffer with protein, detergents and antimicrobial. [Value on vial label]
Low NSB Sample Diluent Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution. See **Assay Design**, page 3.
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

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

ASSAY DESIGN AND SET-UP

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.

Antibody Stability
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; diluted samples are stable refrigerated for several weeks.

Assay Design
Review Interpretation of Results (p5-7) 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 1:250 or greater dilution for human serum with normal levels of IgG and IgM.
- Run the **Anti-Pertussis ACT Positive Control**; value is on the vial label.
- 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, p. 5).

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.

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.

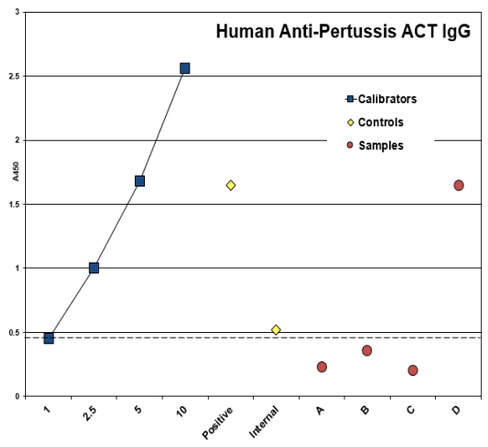
INTERPRETATION OF RESULTS

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-ACT 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 1:250 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from ACT 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 – serum showing natural reactivity to ACT; value is on the vial label. This Control can be used to assess reproducibility and 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:

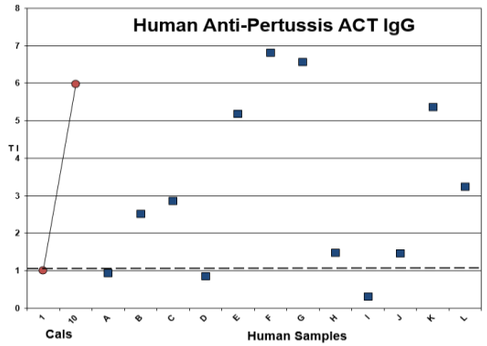
- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Human Serum/Plasma IgG

A panel of human serum/plasma of unknown history was tested for anti-ACT IgG (1:250 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Anti-Pertussis ACT IgG: seven NHS/P were positive at 1:250 dilution (clearly above the 1.0 threshold); one was negative (below the threshold); four samples (A,D,H,J) were borderline.
Notes:

1. **Positives** may be due to prior infection and/or vaccination with *B. pertussis*.
2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) **increase dilution** (e.g., 1:500) to lower the signals of borderline positives to negative, b) **decrease dilution** (e.g., 1:100) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).
3. When a significant portion of the positives are **>5** Index, it may be more useful to run dilution curves to calculate titers (see **Method C**, next page).

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:

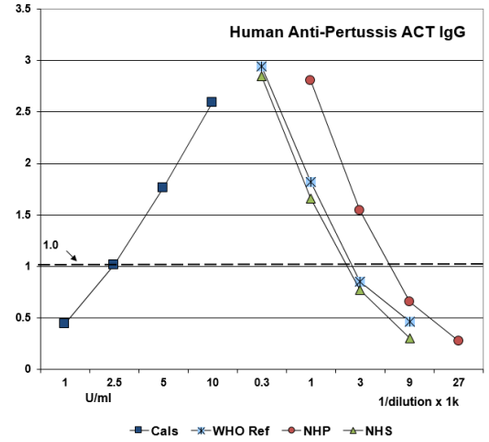
1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.

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.

INTERPRETATION OF RESULTS (cont)

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: **2.5 U**/ml. The Calibrator titer value can be used to normalize between-assay sample titer values.

WHO Reference 1st RR: preparation 06/142, human anti-pertussis established as immunoassay reference. Titer: **2.5 k .**

NHP: a human plasma of unknown history. Titer: **5.9 k**

NHS: a human serum of unknown history. Titer: **2.25 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.

PRECAUTIONS AND SAFETY INSTRUCTIONS

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. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

Instruction Manual No. M-960-510-AHG

Human Anti-Pertussis ACT (Adenyl Cyclase Toxin) IgG

ELISA Kit Cat. No. 960-510-AHG

For Quantitation of Anti-*B. Pertussis* ACT IgG in Serum or Plasma

For research use only, not for diagnostic or therapeutic use.

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 W • San Antonio• Texas 78249 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777
www.4adi.com
service@4adi.com

ELISA Kit Components	Amount	Part #
Pertussis ACT Coated Strip Plate	8-well strips (12)	960-511
Anti-Pertussis ACT Positive Control	0.65 ml	960-512PC
Anti-Pertussis ACT Calibrator 1 U/ml	0.65 ml	960-512B
Anti-Pertussis ACT Calibrator 2.5 U/ml	0.65 ml	960-512C
Anti-Pertussis ACT Calibrator 5 U/ml	0.65 ml	960-512D
Anti-Pertussis ACT Calibrator 10 U/ml	0.65 ml	960-512E
Anti-Human IgG HRP Conjugate (100X)	0.15 ml	H-HuG.2a11
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-960-510-AHG