INTENDED USE

The **Human Anti-B. Pertussis Toxin (PTX) IgM** ELISA Kit detects and quantifies pertussis toxin/toxoid-specific IgM in serum or plasma of vaccinated or immunized humans. This immunoassay 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 (RUO), not for diagnostic 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 pertussis toxin. Pertussis 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.

Monitoring the efficacy of vaccines by determining the anti-pertussis levels in the host, including for clinical trials using new formulation of vaccines, is often required. The ADI Anti-Pertussis Toxin (PTX) ELISAs will quantify antibodies produced by vaccines as well as from infection with the toxin-producing organisms.

PRINCIPLE OF THE TEST

The **Human Anti-Pertussis Toxin/toxoid IgM** ELISA kit is based on the binding of human anti-pertussis toxin IgM in samples to pertussis toxin immobilized on the microwells, and anti-pertussis toxin IgM antibody is detected by anti-human IgM specific antibody conjugated to HRP (horseradish peroxidase) enzyme. 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-pertussis toxin IgM present in the sample. Stopping Solution is added to terminate the reaction and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human IgM antibody in samples is calculated relative to anti-pertussis toxin calibrators.

PRODUCT SPECIFICATIONS

Specificity

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

Assay Sensitivity

The pertussis toxin coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate anti-pertussis toxin IgM from background (non-antibody) signal with human serum samples diluted 1:200.

Calibrator Values

The calibrators are dilutions of antibody reactive to pertussis toxin. Values are assigned as arbitrary anti-pertussis toxin 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 IgM-HRP Conjugate Concentrate (100x) Part: H-HuM.2a11, 0.15ml	Peroxidase conjugated anti-human IgM 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
PTX Coated Strip Plate	960-131	8-well strips (12)	Coated with *B. pertussis* toxin, and post-coated with stabilizers.
Anti-PTX Calibrators			
10 U/ml 25 U/ml 50 U/ml 100 U/ml	960152B 960152C 960152D 960152E	0.65ml 0.65ml 0.65ml 0.65ml	Four (4) vials, each containing anti-pertussis toxin; in buffer with antimicrobial.
Anti-Pertussis Toxin Positive Control	960-152-PC	0.65ml	Anti-pertussis toxin; diluted in buffer with protein, detergents and antimicrobial. [Value range on 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.

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 (LNSD)**, which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same day as the assay.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design

Review Interpretation of Results (p. 5-7) 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 **10 U/ml Calibrator**. This is usually 1:200 or greater dilution for human serum with normal levels of IgG and IgM.
- Run the **Anti-Pertussis Toxin Positive Control**; value range is on the 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: **100 U/ml** should give a high signal (>1.5 OD); **10 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 1-5 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 IgM HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

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.

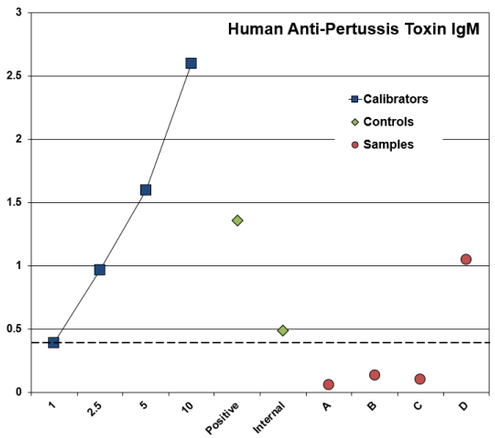
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **10 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-pertussis toxin IgM, from either natural infection or vaccination, is controlled so that the **10 U/ml Calibrator** represents a threshold OD for most true positives in human serum diluted to 1:200 or greater. Visual inspection of the data in the above graph shows the following:

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

10 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 – antiserum from vaccination with pertussis toxin; value range 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 (1:250) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

The 10 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

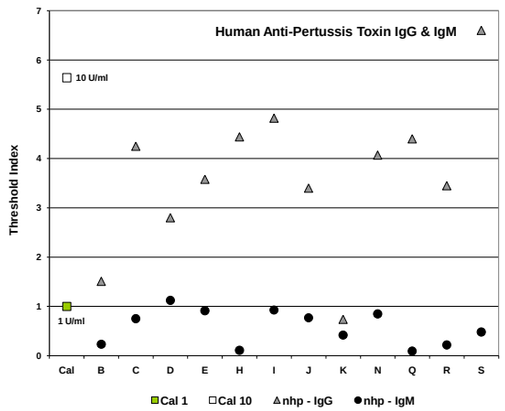
- Divide each Sample net OD by the 10 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Human Serum/Plasma IgG & IgM

A panel of human serum/plasma of unknown history were tested for anti-pertussis toxin IgG (1:500 dilution) and IgM (1:250 dilution). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Anti-Pertussis IgG: most NHS/P were positive at 1:500 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).

Anti-Pertussis IgM: no NHS/P were positive at 1:250 dilution.

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.

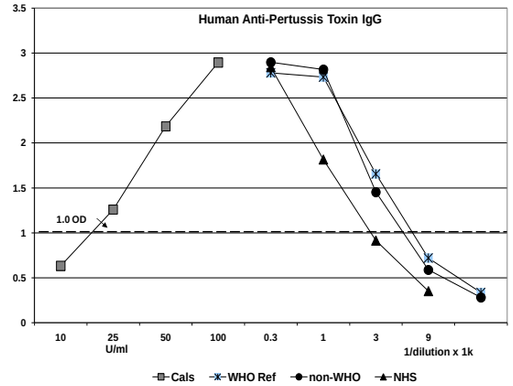
A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

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

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: **17.5 U/**ml. The Calibrator titer value can be used to normalize between-assay sample titer values.
Note: **1** U/ml = **0.93** mIU/ml WHO Reference.

WHO Reference 1st RR: preparation 06/142, human anti-pertussis (106 IU/ml), established as immunoassay reference. Titer: **6.5 k** (16.3 mIU/ml)

Non-WHO Reference: (NIBSC 89/530) human anti-pertussis sera from infection or vaccination, established as immunoassay reference. Titer: **5.3 k**

NHS: a human serum (NHS) of unknown history. Titer: **2.7 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.

Instruction Manual No. M-960-195-PHM

Human Anti-*B. Pertussis* Toxin/Toxoid (PTX) IgM

Cat. #960-195-PHM, 96 tests

For Quantitation of Anti-Pertussis Toxin/Toxoid IgM in Serum

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

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ELISA Kit Components	Amount	Part #
PTX coated Strip Plate	8-well strips (12)	960-131
Anti-Pertussis Toxin Positive Control	0.65 ml	960-152-PC
Anti-Pertussis Toxin Calibrator 10 U/ml	0.65 ml	960-152B
Anti-Pertussis Toxin Calibrator 25 U/ml	0.65 ml	960-152C
Anti-Pertussis Toxin Calibrator 50 U/ml	0.65 ml	960-152D
Anti-Pertussis Toxin Calibrator 100 U/ml	0.65 ml	960-152E
Anti-Human IgM HRP Conjugate (100X)	0.15 ml	H-HuM.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-960-195-PHM