# SeroPertussis™ Toxin IgG

Enzyme Linked Immunosorbent Assay (ELISA) for the quantitative determination of specific IgG antibodies to ***Bordetella Pertussis* Toxin** in human serum

**Instruction Manual**

Test kit for 96 determinations
(Catalog No.1231-01)

For ***In Vitro*** Diagnostic Use
For professional use only
Store at 2-8°C. **Do Not Freeze**

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**Intended Use**
SeroPertussis™ Toxin IgG kit is a quantitative Enzyme Linked Immunosorbent assay (ELISA) for the determination of specific IgG antibodies to *Bordetella pertussis* Toxin in human serum or plasma.

For ***In Vitro*** Diagnostic Use

## Introduction

Whooping Cough (Pertussis) is a highly contagious bacterial respiratory tract infection, caused by *Bordetella pertussis* Gram-negative bacilli, an exclusively human pathogen which can affect people of all ages. Transmission of the organism takes place through air droplets produced with cough or sneezes as a result of close direct contact with an infected person. Ninety percent of susceptible household contacts will develop clinically relevant disease following exposure. Despite extensive childhood immunization for several decades, pertussis remains one of the world's leading causes of vaccine preventable deaths (1, 2). The most severe disease occurs in unimmunized infants and young children who are the most vulnerable group with the highest rates of complications and death. The disease is usually milder in adolescents and adults who constitute a reservoir and are a source of spread to young children. (3).

**Epidemiology:** Pertussis is an endemic disease, but epidemics occur every 3 – 5 years. In the USA, 5000 – 7000 cases are reported each year. It has been reported that 21% of the adults in the United States with prolonged cough (lasting >2 weeks) had pertussis (4). Estimates from WHO suggest that in 2008 about 16 million cases of pertussis occurred worldwide, 95% of which were in developing countries, and that about 195,000 children died from this disease (5). Since 2011, increases in the number of cases of pertussis have been repeatedly reported in different regions of the world, even in those with sustained high vaccination coverage. In Europe the situation evolves similarly with many countries observing an increment in cases, mostly in infants, adolescents and adults (6).

**Immunization & Vaccination:** Whereas little is known about the duration of protection following pertussis vaccination in developing countries, several studies in the industrialized world show that protection wanes after 4–12 years (7, 8). Infection in vaccinated persons causes a milder non-specific disease, without the classical clinical stages of whooping cough and is seen in only 6% of such cases; instead the illness is characterized by a non-specific, prolonged cough, lasting several weeks to months. Because of these atypical symptoms, pertussis is under-diagnosed in adults and adolescents, who may be the reservoirs for infection of unvaccinated infants (4, 6, 9).

**Management:** Early anti-microbial treatment will reduce the severity of the symptoms and limit the period of transmission. Prompt identification of the cases may help to prevent un-vaccinated or under-vaccinated persons from being infected by vaccination or by anti-microbial prophylaxis.

**Pertussis Diagnosis:** Laboratory diagnosis of Pertussis can be performed either directly (polymerase chain reaction or culture), or indirectly by serological tests which measure the specific antibody response. Since the bacteria reside in the upper respiratory tract during the first three weeks of the infection it can be detected during this period only by direct methods (10, 11, 12).
Serological ELISA based tests aimed at detection of antibodies to Pertussis Toxin are recommended, as such methods can differentiate between *B. pertussis* and *B. parapertussis* infection (13). For serological diagnosis, a significant change of ≥2-fold in anti-pertussis toxin antibodies (increase or decrease in IgG levels) in paired sera would be the most reliable indication for current acute infection in the absence of recent vaccination within the past year (13, 14). However, due to various reasons, a single-point serology test is most commonly practiced (13, 14, 15, 16). Concentrations of antibodies to *B. pertussis* antigens should be quantitatively expressed in International Units per milliliter (IU/ml) as reference preparations are available.

The SeroPertussis™ Toxin IgG utilizes purified pertussis toxin as an antigen, allowing quantitative determination of IgG antibodies to Pertussis Toxin according to the first International WHO Standard (WHO International Standard Pertussis Antiserum, human, 1st IS NIBSC Code 06/140) (17).

## Principle of the Test

- SeroPertussis™ Toxin microtiter plates are coated with enriched fraction of highly purified *Bordetella pertussis* Toxin.
- The serum to be tested is diluted 1/101 and incubated in the SeroPertussis™ Toxin plate. In this step *B. pertussis* Toxin specific antibodies are bound to the immobilized antigens.
- Non-specific antibodies are removed by washing.
- Anti-human IgG conjugated to horseradish peroxidase (HRP) is added. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex.
- Unbound conjugate is removed by washing.
- TMB-substrate is added and is hydrolyzed by the peroxidase, yielding a blue solution of the reduced Substrate.
- Upon the addition of the stop solution, the blue color turns yellow and the absorbance should be read by an ELISA reader at a wavelength of 450/620nm.
- The absorbance is proportional to the levels of the specific antibodies that are bound to the coated antigens.

## Summary of Procedure: Manual/ Automation*

Add 100µl of each Ready-to-Use (RTU) calibrator C30, C60, C120, and 100µl of Negative Control, Positive Control and specimens diluted 1/101 to microtiter plate wells coated with specific immunodominant *B. pertussis* Toxin proteins

↓

Cover plate and incubate 1h at 37ºC at 100% humidity

↓

Wash 5 times with Wash Buffer

↓

Add 100µl of RTU-HRP Conjugate

↓

Cover plate and incubate 1 h at 37ºC at 100% humidity

↓

Wash 5 times with Wash buffer

↓

Add 100µl of TMB Substrate

↓

Cover plate and incubate 15min at room temperature

↓

Add 100 µl of Stop Solution

↓

Read absorbance at 450/620nm

↓

Calculate and interpret results

****Automation procure:***
***Sample incubation:*** *25 minutes at RT followed by an additional incubation of 35 minutes at 35°C.*
***Conjugate incubation:*** *15 minutes at RT followed by an additional incubation of 35 minutes at 35°C.*
***Wash steps:*** *5 wash cycles.*

## Kit contents: for Manual use and Automated use

**Test kit for 96 Determinations; Cat. No. 1231-01**

1. ***B. pertussis* Toxin Antigen Coated Microtiter Plate:** 96 break apart wells (8x12) coated with *Bordetella pertussis* purified Toxin antigen, packed in an aluminum pouch containing a desiccant card.
   **1 Plate**
2. **Concentrated Wash Buffer (20X):** A PBS - Tween buffer. Contains less than 0.05% proclin as a preservative.
   **1 bottle, 100 ml**
3. **Serum Diluent:** A ready-to-use buffer solution contains less than 0.05% proclin as a preservative.
   **2 bottles, 60 ml**
4. **Positive Control:** A ready-to-use *B. pertussis* Toxin IgG positive human serum. Contains a mercury-free and azide-free preservatives.
   **1 Vial, 2 ml**
5. **Negative Control:** A ready-to-use *B. pertussis* Toxin IgG negative human serum. Contains a mercury-free and azide-free preservatives.
   **1 Vial, 2 ml**
6. **C30 Calibrator:** A ready-to-use calibrator containing 30 IU/ml (arbitrary international units) of human specific IgG antibodies to *B. pertussis* Toxin Contains a mercury-free and azide-free preservatives.
   **1 Vial, 2 ml**
7. **C60 Calibrator:** A ready-to-use calibrator containing 60 IU/ml (arbitrary international units) of human specific IgG antibodies to *B. pertussis* Toxin. Contains a mercury-free and azide-free preservatives.
   **1 Vial, 2 ml**
8. **C120 Calibrator:** A ready-to-use calibrator containing 120 IU/ml (arbitrary international units) of human specific IgG antibodies to *B. pertussis* Toxin. Contains a mercury-free and azide-free preservatives.
   **1 Vial, 2 ml**
9. **HRP-Conjugate :**A ready-to-use Horseradish peroxidase (HRP) conjugated anti-human IgG ( $\gamma$ chain specific). Contains a mercury-free and azide-free preservatives.
   **1 Bottle, 15 ml**
10. **TMB-Substrate:** A ready-to-use solution. Contains 3, 3', 5, 5' - Tetramethylbenzidine as a chromogen and peroxide as a substrate.
    **1 Bottle, 16 ml**
11. **Stop Solution:** A ready-to-use solution. Contains 1M $H_2SO_4$.
    **1 Bottle, 16 ml**
12. **Plate Cover:** **1 unit**
13. **Instruction Manual:** **1**
14. **CD** **1**

## Materials Required But Not Supplied

1. Clean test tubes for dilution of patient's sera.
2. Adjustable micropipettes and multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
3. One liter volumetric flask.
4. One 50ml volumetric cylinder.
5. Wash bottle.
6. Absorbent paper.
7. Vortex mixer
8. A 37°C water bath with a lid, or a moisture chamber placed in a 37°C incubator.
9. ELISA-reader with a 450 and 620nm filters.
10. Distilled or double deionized water.

## Warning and Precautions

**For *In Vitro* Diagnostic Use**

1. This kit contains human sera which have been tested by FDA and CE approved techniques, and found to be negative for HBsAg and for HCV and HIV antibodies.

Since no known method can offer complete assurance that products derived from human blood do not transmit infection, all human blood components supplied in this kit must be handled as potentially infectious serum or blood according to the recommendations published in the CDC/NIH manual "Biosafety in Micro Biological and Biomedical Laboratories, 1988".

2. TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
3. All the components of this kit have been calibrated and tested by lot. It is not recommended to mix components from different lots since it might affect the results.
4. Diluted sulfuric acid (1M $H_2SO_4$) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.

## Storage and Shelf -Life of Reagents

1. All the reagents supplied should be stored at 2-8°C. The unopened reagents vials are stable until the expiration date indicated on the kit pack. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents. **DO NOT FREEZE!**
2. Once the kit is opened, its shelf life is 90 days.
3. Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. Crystals may form in the 20x concentrated Wash Buffer during cold storage, this is perfectly normal. Redissolve the crystals by warming the buffer to 37°C before diluting. Once diluted, the solution may be stored at 2-8°C up to twenty one days.

## Serum Collection

Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbid or contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.

## Specimens Storage

Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If longer storage period is anticipated, aliquot and store the specimens below -20°C. Avoid repeated thawing and freezing.

## Test Procedure for Manual Use

**A. Preparation of Reagents**

1. Bring all components and the clinical specimens to be tested to room temperature. Mix gently the calibrators (C30, C60, C120), Negative Control, Positive Control and the clinical specimens before use.
2. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: Three wells of calibrators (C30, C60, C120) and one well of Negative Control. Positive Control testing is optional.
3. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96-well frame.
   Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of wash buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

**B. Incubation of sera samples and controls**

5. Dilute each patient serum 1/101 with the supplied Serum Diluent as follows: Add 10 µl of patient serum to 1000µl of Serum Diluent.
6. Dispense 100µl of each of the three calibrators C30, C60, C120), Negative Control, Positive Control and of serum samples into separate wells of the test strip.
7. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
8. Discard the liquid content of the wells.
9. **Washing step:**
   **Manual Wash:**
   Fill each well with wash buffer up to the end of the well and discard the liquid, repeat this step for a total of 5 washing steps.
   **Automated Wash:**
   Fill each well with 350ul of wash buffer and discard the liquid, repeat this step for a total of 5 washing steps.
10. Dry the strips and frame by gently tapping them over clean absorbent paper.

**C. Incubation with conjugate**

11. Dispense 100µl of ready-to-use HRP- Conjugate into each well.
12. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
13. Discard the liquid content and wash as described in steps 9-10.

**D. Incubation with TMB Substrate**

14. Dispense 100µl TMB Substrate into each well, cover the strips with a plate cover and incubate at room temperature for **15 minutes.**
15. Stop the reaction by adding 100µl of stop solution (1M $H_2SO_4$) to each well.

**E. Determination of Results**

16. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

- ***Note****: Any air bubbles should be removed before reading. The bottom of the ELISA plate should be carefully wiped.*

## Test Procedure for Automated Use

***The vials and reagent's volume have been adapted for automation applications.***

### A. Preparation of Reagents

1. Bring all components and the clinical specimens to be tested to room temperature. Mix well the calibrators (C30,

C60, C120), Negative Control, Positive Control and the clinical specimens before use.
2. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: Three wells of calibrators (C30, C60, C120) and one well of Negative Control. Positive Control testing is optional.
3. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
4. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of wash buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

## B. Incubation of sera samples and controls

5. Dilute each patient serum 1/101as follows:
   - Dispense 750µl of Serum Diluent to each sample tube.
   - Aspirate 250µl of Serum Diluent and 10µl of patient serum.
   - Add the 260µl (1:26 pre-diluted sample) to each sample tube (final volume of 1010µl in each sample tube).
6. Dispense 100µl each of Negative Control, Positive Control, the three calibrators (C30, C60, C120) and 1:101 diluted serum samples into separate wells of the test strip.
7. Incubate for 25 minutes at room temperature (22-28°C). Time incubation is taken starting from dispensing the first vial, as indicated in the automation program: "*Time incubation from start of previous assay step*". Following the incubation at 22-28°C continue to incubate for 35 minutes at 35°C.
8. *Eliminate assay drift caused by this operation.*
9. **Washing step:** Perform 5 X 500µl wash cycles using Savyon's Wash Buffer.
10. Perform 2 aspiration cycles to aspirate residual fluid from the vial, as indicated in the automation program: *"Perform 2 aspirate cycles no aspirate sweep. Partial plate mode: maintain full plate time"*

## C. Incubation with conjugate

*Each vial of HRP Conjugate can only be used twice*

11. Dispense 100µl of Ready-to-Use HRP-conjugate into each well.
12. Incubate for 15 minutes at room temperature (22-*28°C).* Time incubation is taken starting from dispensing the first vial, as indicated in the automation program: "*Time incubation from start of previous assay step*". Following the incubation at 22-28°C continue to incubate for 35 minutes at 35°C.
13. Wash as described in steps 8-9.

## D. Incubation with TMB – Substrate

14. Dispense 100µl TMB-Substrate into each well and incubate at room temperature (22-28°C) for **15 minutes.** Time incubation is taken starting from dispensing the first vial, as indicated in the automation program: "*Time incubation from start of previous assay step*"
15. Stop the reaction by adding 100µl of Stop Solution (1M $H_2SO_4$) to each well.

## E. Determination of Results

16. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction

***Please note that each automation machine has specific technical commands. Please implement Savyon's automation procedure for this kit on the operation protocol of your automation machine.***

## Test Validation

The following criteria must be met for the test to be valid. If these criteria are not met, the test should be considered invalid and should be repeated.

1. C120: OD ≥ 1.3
   C60: OD ≥ 0.9
   C30: OD ≥ 0.5
2. Ratio OD C120 / OD C60: >1.3
3. Ratio OD C60 / OD C30: >1.3
4. **Optional:** Positive Control: > 100 IU/ml (See chapters– Calculation and interpretation of Test Results)
5. Negative Control: <15 IU/ml (See chapters – Calculation and interpretation of Test Results)

## Calculation of Test Results

***PLEASE USE "MASTER INTERPRETATION FOR SeroPT" FILE (SEE ENCLOSED DISK*) FOR QUICK CALCULATION AND INTERPRETATION OF TEST RESULTS.**

**CALCULATION OF TEST RESULTS CAN BE ALSO PERFORMED BY:**

**1. Manual method, using a squared graph paper:**

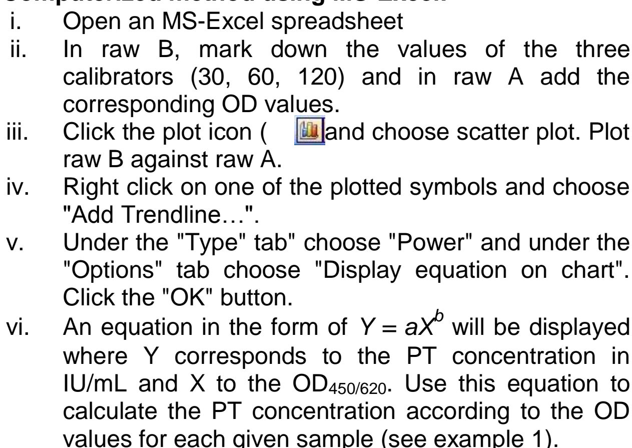
i. Plot the absorbance values (OD) of the 3 calibrators (C30, C60 and C120) on X axis versus their concentration (IU/ml) on Y axis.

ii. Using the standard curve, interpolate the concentration of the tested sample values (in IU/ml) from each absorbance measured (see example 1).

**2. Computerized method using MS-Excel:**

i. Open an MS-Excel spreadsheet

ii. In raw B, mark down the values of the three calibrators (30, 60, 120) and in raw A add the corresponding OD values.

iii. Click the plot icon (  and choose scatter plot. Plot raw B against raw A.

iv. Right click on one of the plotted symbols and choose "Add Trendline…".

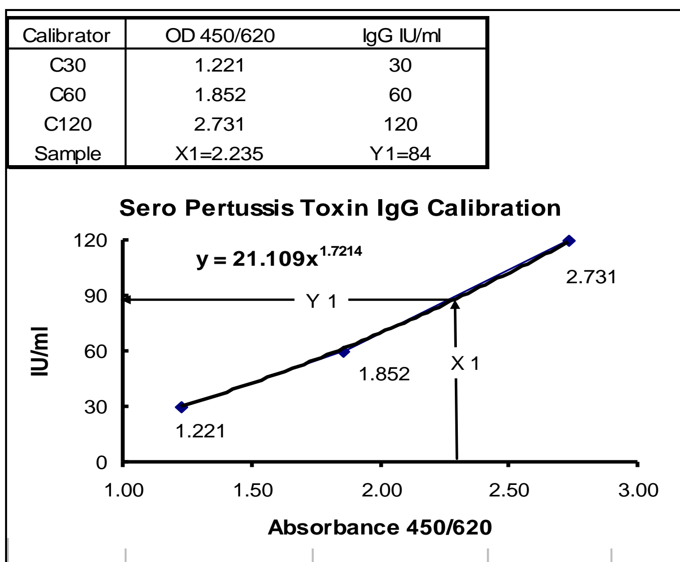
v. Under the "Type" tab" choose "Power" and under the "Options" tab choose "Display equation on chart". Click the "OK" button.

vi. An equation in the form of $Y = aX^b$ will be displayed where Y corresponds to the PT concentration in IU/mL and X to the $OD_{450/620}$. Use this equation to calculate the PT concentration according to the OD values for each given sample (see example 1).

**Example 1. Interpolation of results:**

Read the absorbance value of the sample (X1) and mark it on the X-axis. Draw a vertical line from this point to the calibration curve, and from the intercept draw a horizontal line to the Y-axis.

Read the concentration in IU/ml of the sample.



| Calibrator | OD 450/620 | IgG IU/ml |
|---|---|---|
| C30 | 1.221 | 30 |
| C60 | 1.852 | 60 |
| C120 | 2.731 | 120 |
| Sample | X1=2.235 | Y1=84 |



Excel: (Y) =21.109*2.235^1.7214

## Interpretation of Results

1. **Validity of sample results:** If $OD_{450/620}$ of a certain sample is ≥ 3.0, it is recommended that the sample will be re-tested at a dilution of 1:404. The result in IU/ml of this particular sample should be then multiplied by a factor of 4.

2. **Cut-off:** According to recent literature and recommendations from reference laboratories across the EU (16, 17), Savyon Diagnostics recommends the following interpretation of results:

| IgG IU/ml | Result | Interpretation |
|---|---|---|
| <40 IU/ml | **Negative** | *No indication for an acute Infection* |
| ≥40 to <100 IU/ml | **Intermediate** | *Possible infection; re-test IgG in 2-4 weeks or test for IgA levels* |
| ≥100 IU/ml | **Positive** | *Indication for an acute infection or recent contact (in absence of recent vaccination)* |

3. **Testing convalescent sera samples:** If diagnosis cannot be established with certainty from a single serum (Intermediate range), but is deemed to be necessary according to the clinical symptoms, Savyon Diagnostics recommends to re-test second (convalescent) serum sample taken 2-4 weeks later (13,15). Recent literature recommends that ≥ 2-fold change in IgG levels is indicative for current acute infection (13, 14).

4. **IgA testing and antibody profiling:** In case of non-availability of a second serum sample and In order to achieve a comprehensive antibodies profile, Savyon Diagnostics recommends also to test IgA levels (13.15):

| IgG | IgA | Interpretation |
|---|---|---|
| Negative | Negative | *No indication of B. pertussis infection (see test limitations)* |
| Intermediate | Negative | *No recent infection* |
| Intermediate or Negative | Positive | *Indication of recent infection* |
| Positive | Negative or Positive | *Indication of recent infection* |

## Test Limitations

1. No single serological test should be used for final diagnosis. All clinical and laboratory data should be taken into account.
2. Samples obtained too early during primary infection may not contain detectable antibodies. If *B. pertussis* is suspected, a second sample should be obtained 2-4 weeks later and tested in parallel with the original sample.
3. When infection is suspected in infants under the age of 6 months, another test should be performed (culture, PCR) since children younger than 6 months rarely develop antibodies.
4. Serological diagnosis for *B. pertussis infection* must not be performed if vaccination took place less than 1 year.

## Performance Characteristics

**Precision**

Intra-assay (within-run) precision:

| Sample | No of Replicates | Mean OD Value | CV% |
|---|---|---|---|
| 120IU/m | 10 | 119 | 3.5 |
| 30IU/ml | 10 | 32 | 7.1 |

Inter-assay (between-run) precision:

| Sample | No of Replicates | Mean OD Value | CV% |
|---|---|---|---|
| 120IU/ml | 10 | 122 | 6.3 |
| 30IU/ml | 10 | 30 | 9.4 |

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|---|---|
| 2 °C – 8 °C | Temperature Limitation |
| | Consult instructions for use |
| IVD | In Vitro Diagnostic Medical Device |
| | Manufacturer |
| EC REP | Authorized European Representative |