

## Related ELISA kits available from ADI (see details at the web site)

### Catalog# ProdDescription

960-210-PHG	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG, 96 tests,
960-120-PHG	Mouse Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA kit,
960-130-PMG	Mouse Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-140-PMM	Mouse Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-150-PRG	Rabbit Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-160-PRM	Rabbit Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-170-PMG	G. pig Anti-B. pertussis toxin/toxoid IgG ELISA kit, 96 tests, Quantitative
960-180-PMM	G. pig Anti-B. pertussis toxin/toxoid IgM ELISA kit, 96 tests, Quantitative
960-200-PHA	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,
960-205-PHA	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgA ELISA kit,
960-210-PHG	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgG ELISA kit,
960-220-PHM	Human Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,
960-225-PHM	Monkey Anti-B. pertussis antigens (Pertussis toxin, FHA and LPS) IgM ELISA kit,
960-230-PGG	Mouse Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-240-PRG	Rabbit Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-250-PHG	Human Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-260-PMG	Monkey Anti-B. pertussis Pertactin IgG ELISA kit, 96 tests
960-300-FMG	Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-310-FMM	Mouse Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96
960-320-FRG	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-330-FRM	Rabbit Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96
960-340-FHG	Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgG ELISA kit, 96
960-350-FHM	Human Anti-B. pertussis Filamentous hemeagglutinin (FHA) IgM ELISA kit, 96
940-100-DHG	Human Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-120-DMG	Mouse Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-125-DMM	Mouse Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-130-DRG	Rabbit Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-135-DRM	Rabbit Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-140-DGG	Guinea Pig Anti-Diphtheria Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
940-145-DGM	Guinea Pig Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
940-150-HFA	Horse Anti-Diphtheria Toxin/Toxoid IgG (Fab2) ELISA kit, 96 tests, Quantitative
940-200-DHG	Human Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,
940-210-DHM	Human Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,
940-220-DMG	Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,
940-225-DMM	Mouse Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,
940-230-DRG	Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgG ELISA kit, 96 tests,
940-235-DRM	Rabbit Anti-CRM197 (Diphtheria Toxin mutant) IgM ELISA kit, 96 tests,
940-245-DKM	Monkey Anti-Diphtheria Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
930-100-TTH	Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-110-TTM	Mouse Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests, Quantitative
930-120-TMA	Mouse Anti-Tetanus Toxin/Toxoid IgA ELISA kit, 96 tests, Quantitative
930-130-TMG	Mouse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-140-TMM	Mouse Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
930-200-TTR	Rabbit Anti-Tetanus Toxin/Toxoid Ig's (G+A+M) ELISA kit, 96 tests, Quantitative
930-210-TRG	Rabbit Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-220-TRM	Rabbit Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
930-310-TGG	G. pig Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-320-TGM	G. pig Anti-Tetanus Toxin/Toxoid IgM ELISA kit, 96 tests, Quantitative
930-410-TKG	Monkey Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-500-HTG	Horse Anti-Tetanus Toxin/Toxoid IgG ELISA kit, 96 tests, Quantitative
930-510-HFA	Horse Anti-Tetanus Toxin/Toxoid IgG-Fab2 ELISA kit, 96 tests, Quantitative

**Pertussis Vaccines:** Trihibit (DTAP/Hib), ActHib (Hib-PRP-T), Daptacel (DTAP), Tripedia (DTAP), Adacel (tetanus, Diphtheria, Acellular Pertussis) - Sanofi Pasteur; PedvaxHib (Hib-PRP-OMP) – Merck; Pediarix (DTAP/HepB/IPV), Infanrix (DTAP), Boostrix (Tetanus, Diphtheria, Acellular Pertussis) - GlaxoSmithKline.

Instruction Manual No. M-960-210-PHG

## Monkey Anti-Bordetella pertussis IgG ELISA Kit

**Cat. # 960-210-PHG, 96 Tests**

**For the detection of Anti-Bordetella pertussis IgG  
In monkey Serum or Plasma**

*For In Vitro Research Use Only*



**India Contact:**

**Life Technologies (India) Pvt. Ltd.**

306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034  
Ph: +91-11-42208000, 42208111, 42208222

Mobile: +91-9810521400

Fax: +91-11-42208444

Email: [customerservice@atzlabs.com](mailto:customerservice@atzlabs.com)

Web: [www.atzlabs.com](http://www.atzlabs.com)



## Anti-Bordetella pertussis antigens IgG ELISA KIT #960-210-PHG

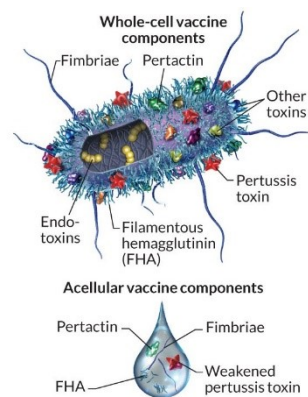
This kit has been designed for the detection of Anti-Bordetella pertussis IgG serum or plasma. For research use only, not for use in diagnostic procedures.

K i t C o m p o n e n t s	C a t . #
B. pertussis antigens coated <b>ELISA strips</b> (96 wells)	960211
<b>Anti-B. Pertussis IgG Calibrator A</b> (1 U/ml), 2 ml	960212A
<b>Anti-B. Pertussis IgG Calibrator B</b> (20 U/ml), 2 ml	960212B
<b>Anti-B. Pertussis IgG Calibrator C</b> (45 U/ml), 2 ml	960212C
<b>Anti-B. Pertussis IgG Calibrator D</b> (150 U/ml), 2 ml	960212D
Controls are diluted serum base containing 0.01% BND as preservative	
<b>Sample Diluent</b> , 60 ml buffer with 0.09% azide	960210-SD
<b>Wash buffer (10X)</b> , 60 ml	960210-WB
<b>Anti-hIgG HRP Conjugate</b> , 15 ml, ready to use	960213
<b>HRP Substrate Soln (TMB)</b> , 15 ml, ready to use	960210-TM
<b>Stop Solution</b> (diluted sulfuric acid), 15 ml	960210-SS
Complete Instruction Manual	M-960210PHG

### Intended use:

ADI's **Anti-Bordetella pertussis IgG** ELISA kit is intended for the detection of Anti-Bordetella pertussis IgG in serum or plasma. For In Vitro Research Use Only (RUO).

### General Information:



Whooping cough is a disease of the respiratory tracts which is caused by Bordetella pertussis bacteria. It is transmitted by airborne infection. The gram-negative Coccobacillus produces a series of biologically active molecules. The different compounds appear either during the pathogenesis or during the process of immunization against pertussis and show different effects. A characterisation has been made for the pertussis toxin (PT), the filamentary haemagglutinin (FHA) and different lipopolysaccharides (LPS). Pertussis shows a high rate of transmission (rates of infection of over 90 % have been found for nonvaccinated household members) and can cause severe diseases, especially for very young children. From 10749 patients under one year between 1980 and 1989 69 % were brought into hospital, 22 % suffered from pneumonia, 0.9 % showed an Encephalopathy and

0.6 % died. For older children and adults (including already vaccinated persons) the infection may be observed by an unspecified bronchitis or inflammation of the upper respiratory tracts. Even asymptomatic cases are quite common.

The serological response following pertussis disease or immunization with pertussis vaccine has been measured with agglutination assays, precipitins, complement fixation

## Interpretation of results:

Most of the data presented here is for information purpose. Therefore, users are suggested to establish their own reference values. This information is derived from human studies as no reference values for monkey samples are available.

U/mL	Interpretation
< 18	negative
18 - 22	equivocal
> 22	positive

The results themselves should not be the only reason for any therapeutic consequences. They have to be correlated to other clinical observations and diagnostic tests.

## PERFORMANCE CHARACTERISTICS

Bordetella pertussis ELISA IgG

Intra-Assay-Precision	5.0 %
Inter-Assay-Precision	4.3 %
Inter-Lot-Precision	2,6 – 4.5 %
Analytical Sensitivity	0,98 U/mL
Recovery	106 – 114 %
Linearity	78 – 124 %

**Cross-Reactivity:** No cross-reactivity to RSV, Adenovirus and Parainfluenza IgG

**Interferences:** No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL und triglycerides up to 5.0 mg/mL

<b>Clinical Specificity</b>	84 %
<b>Clinical Sensitivity</b>	100 %

## Specificity of the Antigens

The coated antigens in this kit contain B. Pertussis antigens. It has B. pertussis toxin, filamentous hemagglutinin (FHA), pertactin (PRN), LPS among others. Therefore this kit will detect antibodies to all major B. pertussis antigens. ADI has other kits that have purified Pertussis toxin or FHA or PRN antigens to investigate the presence of specific antibodies (see the list on page 8).

## Species reactivity

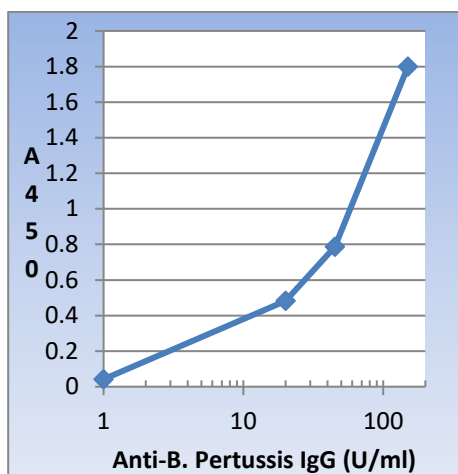
This kit is designed for monkey serum or plasma. ADI also has kits for mouse, rabbit and other species.

**References:** Chodorowska, M. (1996) Med. Dosw. Microbiol., 48:15

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples (U/ml)	Net Mean $A_{450\text{ nm}}$	Calculated Conc. (U/ml)
A1, A2	<b>Calibrator A</b> (1 U/ml)	0.042	
B1, B2	<b>Calibrator B</b> (20 U/ml)	0.483	
C1, C2	<b>Calibrator C</b> (45 U/ml)	0.786	
D1, D2	<b>Calibrator D</b> (150 U/ml)	1.789	

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values (:/2\_ADI\_ELISA\_Graphs)



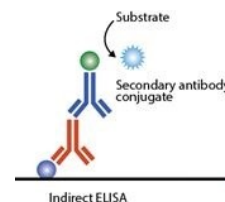
## INTERPRETAION AND CALCULATION OF RESULTS

The obtained OD of the standards (y-axis, linear) are plotted against their concentration (x-axis, logarithmic) either on semi-logarithmic graph paper or using an automated method. A good fit is provided with cubic spline, 4 parameter logistics or Logit-Log. For the calculation of the standard curve apply each signal of the standards (one obvious outlier of duplicates might be omitted and the more plausible single value might be used). The concentration of the samples can be read from the standards curve. The initial dilution has been taken into consideration when reading the results from the graph. Results of samples of higher predilution (1:100) have to be multiplied with the dilution factor. Samples showing concentrations above the highest standard have to be diluted as described in "Assay

and enzyme-linked immunosorbent assay (ELISA). Enzyme-linked immunosorbent assays, in which Bordetella antigen (containing toxin, FHA and LPS and standardized in U/ml) is bound to a solid phase support, are sensitive, easy to perform and can be used both to determine seropositivity with a single serum and to indicate recent Bordetella infection by determination of IgM and IgA identification of the dyed bacteria in sputum. Recently specific antigens have been prepared either by purification of natural material or by recombinant methods.

Additional ELISA kits to detect the Mycobacterium tuberculosis virus antibody in mouse and other species are also available for research. These kits should be useful to determine the M. tuberculosis antibodies due to natural infection or upon vaccination with BCG vaccine.

## PRINCIPLE OF THE TEST



Anti-Bordetella pertussis IgG ELISA kit is based on binding of antibody from serum samples to M. tuberculosis antigens immobilized on microtiter wells. After a washing step, anti-IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of IgG present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of IgG in samples is calculated compared with the absorbance of the supplied negative and positive controls.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000  $\mu$ l) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

## PRECAUTIONS

This ELISA test is intended for *in vitro research* use only. The reagents contains Monkey serum and preservative; necessary care should be taken when disposing solutions. Monkey sera are shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v).

## SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. EDTA/Heparin plasma can also be used.

### Preparation of the reagent:

**Dilute wash buffer (1:10)** with distilled water (**60 ml stock in total of 600 ml water**). store at 4oC. If stock shows crystal then it can be dissolved by bringing to room temp or slight warming.

### STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions. Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

### TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE).

1. Label, and secure the microtiter well strips to be used on the plate.  
**Dilute** samples (1:101) in sample diluent. Controls provided in the kit are already pre-diluted.
2. Pipet **100 ul of sample** diluent (for use as blanks), pre-*diluted* negative, positive controls, and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently for 5-10 seconds, cover the plate and incubate for **60 minutes** at room temp (24-28oC).
3. Aspirate and **wash the wells 3 times** with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 ul of antibody-enzyme conjugate** into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp.
5. Aspirate and wash the wells 3 times as above.
6. Dispense **100 ul TMB substrate per well**. Mix gently for 5 seconds. Cover the plate and incubate at room temp in the dark. for **20 minutes**. **Blue color** develops in positive wells.
7. Stop the reaction by adding **100 ul** of stopping solution to all wells at the same timed intervals . Mix gently for 5-10 seconds. **Blue color turns yellow**. Measure the absorbance at 450 nm using an ELISA reader.

### Limitations, Precautions and General Comments:

- Only for in-vitro use!
- Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, they should not be mixed among one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.