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

Catalog#	ProdDescription
960-110-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-960-110-PHG

Human Anti-Bordetella pertussis IgG ELISA Kit

Cat. # 960-110-PHG, 96 Tests

For the detection of Anti-Bordetella pertussis IgG In Serum/Plasma

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 West• San Antonio• Texas 78249 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email: service@4adi.com
Web Site: www.4adi.com

Anti-Bordetella pertussis IgG ELISA KIT #960-110-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.

Kit Components	Cat.#
B. pertussis antigens coated **ELISA strips** (96 wells)	960111
Calibrator A, Negative Control, 2 ml	960112A
Calibrator B, Cut-off Control, 3 ml	960112B
Calibrator C, Positive Control, 2 ml	960112C
Controls containing 0.01% MIT as preservative	
Sample Diluent, 100 ml	960110-SD
Wash buffer (20X), 50 ml	960110-WB
Anti-hIgG **HRP Conjugate**, 20 ml, ready to use	960113
HRP Substrate Soln (TMB) , 15 ml, ready to use	960110-TM
Stop Solution (diluted sulfuric acid), 15 ml	960110-SS
Complete Instruction Manual	M-960110PHG

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 gramnegative 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 filamentery haemagglutinine (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 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.

Interpretation of results:

Most of the data presented here is for information purpose. Therefore, users are suggested to establish their own reference values.

U	Interpretation
< 9U	negative
9-11U	equivocal
> 11U	positive
10U	Cut-Off

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

Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value < 0.100
- **Negative Control**: Absorbance value < 0.200 and < Cut-off
- **Cut-off Control**: Absorbance value 0.150 – 1.300
- **Positive Control**: Absorbance value > Cut-off

If these criteria are not met, the test is not valid and must be repeated.

Expected Values:

In an in-house study apparently healthy subjects showed the following results:

Ig Isotype	n	Interpretation		
		positive	equivocal	Negative
IgG	54	79.6 %	7.4 %	13.0 %

PERFORMANCE CHARAVEREISTICS

Bordetella pertussis ELISA IgG

Intra-Assay-Precision	3.55 %
Inter-Assay-Precision	14.08 %
Sensitivity	98.31%
Specificity	93.02%

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

Interferences: No interferences to bilirubin up to 0.5 mg/mL, hemoglobin up to 10.0 mg/mL und triglycerides up to 5.0 mg/mL

Species reactivity

This kit is designed for human 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 $A_{450\ nm}$
A1, A2	**Std. A** negative control	0.003
B1, B2	**Std. B** Cut-off	0.576
C1, C2	**Std. C** positive control	1.417

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

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 "Test procedure"

The Cut-off is the mean absorbance value of the Cut-off Control determinations.
Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

Results in Units [U]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-Off}} = [\text{Units} = \text{U}]$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ U (Units)

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 Bordetella antigen 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 µl) 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 human serum and preservative; necessary care should be taken when disposing solutions. Human 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.

TMB (substrate), H2SO4 (stop solution), All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (diluted sulfuric acid).

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^{O}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.

Sample Dilution
Before assaying, Samples should be diluted 1+100 with IgG Sample diluent. Dispense 10 µL sample and 1 mL sample diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

Preparation of the reagent:

Dilute wash buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water**. s**tore at 4^{o}C. 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^{o}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 (1:100) in sample diluent. **Calibrators provided in the kit are ready-to-use**.

2. Pipet **100 µL** each of the **diluted** (1:100) samples and the **ready-to-use** calibrators respectively into the wells. Leave one well empty for the substrate blank.

3. Mix gently for 5-10 seconds, cover the plate and incubate for **60 minutes** at 37°C.

4. 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.

5. Add **100 ul of enzyme conjugate** into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **30 minutes** at room temp. (20-25 °C).

6. Aspirate and wash the wells 3 times as in step 4.

7. Dispense **100 ul TMB substrate per well**. Mix gently for 5 seconds. Cover the plate and incubate at **room temp** (20-25 °C) **in the dark** for **15 minutes**. **Blue color** develops in positive wells.

8. Stop the reaction by adding **100 ul** of stop 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 within 30 minutes.

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.