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

Instruction Manual No. M-940-100-DHG

Human Anti-Diphtheria Toxoid IgG

ELISA KIT

Cat. No. 940-100-DHG, 96 Tests

For detecting human IgG antibody to Diphtheria toxoid in serum, plasma or other biological fluids

For In Vitro Research Use Only (RUO)

ALPHA DIAGNOSTIC INTERNATIONAL

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(210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

Human Anti-Diphtheria Toxoid IgG Elisa kit# 940-100-DHG

Kit Contents: (reagents for 96 tests)

Components	
Diphtheria Toxoid antigen coated microwell strip plate (8x12 or 96 wells);#940101	1 plate
Diphtheria **Std. A**, 0 IU/mL, 2 ml; #940102A	1 vial
Diphtheria **Std. B**, 0.015 IU/mL, 2ml, #940102B	1 vial
Diphtheria **Std. C**, 0.075 IU/mL, 2ml; #940102C	1 vial
Diphtheria **Std. D**, 0.150 IU/mL, 2 ml;, #940102D	1 vial
Note: Stds (ready to use) are calibrated against WHO, 2012	
Sample Diluent 100 ml, #940100-SD	1 bottle
Anti-human IgG-**HRP Conjugate**, 20 ml, #940103	1 bottle
HRP **substrate Solution**, 15 ml # 940100-TMB	1 bottle
Wash buffer (20X), 50 ml, dilute 1:19 with water #940100-WB	1 bottle
Stop solution (ready-to-use), 15 ml, #940100ST	1 bottle
Complete Instruction Manual; M-940-100-DHG	1

Intended Use

Diphtheria IgG antibody ELISA kit has been designed for the detection of IgG class antibodies against Diphtheria Toxoin/Toxoid in human serum and plasma. For research use only.(RUO), not for use in diagnostic procedures

Diphtheria (Greek diphthera)—"pair of leather scrolls") is an upper respiratory tract illness characterized by sore throat, low fever, and an adherent membrane on the tonsils, pharynx, and/or nasal cavity. It is caused by *Corynebacterium diphtheria*, an aerobic Gram-positive bacterium. Diphtheria causes the progressive deterioration of myelin sheaths in the central and peripheral nervous system leading to degenerating motor control and loss of sensation. Diphtheria is a contagious disease spread by direct physical contact or breathing the aerosolized secretions of infected individuals. Children represent a large majority of these cases and fatalities. Common diphtheria has largely been eradicated in industrialized nations through widespread vaccination. DPT (Diphtheria–Pertussis–Tetanus) vaccine is recommended for all school aged children. Diphtheria toxin consists of a single polypeptide ~58 Kda. Proteolysis yields two fragments (A ~21 kda and B ~37 Kda) which are held together by a disulfide bond. The toxin enters the host cell and is hydrolysed by a trypsin-like protease to give a fragment with enzymatic activity. CRM197 is a non-toxic mutant containing a single amino acid substitution of Glu to Arg. Diphtheria Toxin/Toxoid and CRM197 are immunologically indistinguishable. CRM197 is used as a protein conjugate of several vaccines. The state of immunity can be monitored by determining the antitoxin IgG.

Diphtheria Vaccines: Pediarix (DTAP/HepB/IPV), Infanrix (DTAP), Boostrix (Tetanus, Diphtheria, Acellular Pertussis) –GlaxoSmithKline; Trihibit (DTAP/Hib), Daptacel (DTAP), Tripedia (DTAP), DT (Pediatric), Td (Adult), DecavacTM (Tetanus/Diphtheria), Adacel (tetanus, Diphtheria, Acellular Pertussis) Sanofi Pasteur.

Interpretation of Results

<0.01 IU/mL	No protective antibody level. Immediate full course of basic immunization is recommended.
0.01 - 0.09 IU/mL	No reliable protection. Immediate booster injection is recommended.
0.1 – 1.0 IU/mL	Reliable protection.
> 1.0 IU/mL	Reliable long term protection:

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
- Standard A: Absorbance value < 0.200
- Standard B: Absorbance value > 0.100
- Standard C: Absorbance value > 0.500
- Standard D: Absorbance value > 1.000

Standard A < Standard B < Standard C < Standard D

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

Performance Characteristics

Intraassay	<3.86%
Interassay	<12.95%
Specificity	100%
Sensitivity	100%
Analytical Sensitivity	0.00092 IU/mL
Measurement range	0.00092 IU/mL – 0.15 IU/mL.

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

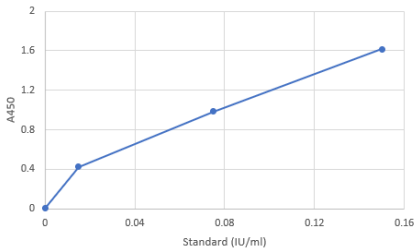
Human and Animal (monkey specificity)

This kit employs anti-IgG-HRP conjugate that reacts with human IgG and not with IgM or IgA. ADI has separate ELISA kits to measure IgM and IgA anti-Diphtheria IgG. This kit is not suitable for animal use. ADI has other similar kits to detect anti-Diphtheria Ig's in animals.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples IU/mL	Mean A_{450nm}
A1, A2	**Std. A** (0)	0.005
B1, B2	**Std. B** (0.015)	0.425
C1, C2	**Std. C** (0.075)	0.984
D1, D2	**Std. D** (0.150)	1.618

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.



CALCULATION OF RESULTS

In order to obtain results in IU/mL plot the (mean) absorbance values of the four standards A-D on (linear/linear) graph paper in a system of cordinates against thier corresponding concentrations (0- 0.150 IU/ml) and draw standard calibration curve (abs value on y-axis & concn on the x-axis)

Read results from this standard curve employing the (mean) absorbance values of each sample.

For the calculation of the satndard mathmatical Point to Point function should be used.

It is necessary to monitor the efficacy of vaccines and determine the anti-Diphtheria Ig levels in patients or for clinical trials using new formulation of vaccines. ADI has developed antibody ELISA kits to determine the efficacy of various existing Diphtheria vaccines and test new vaccines. ADI has also introduced industry's first ELISA for direct testing of Diphtheria Toxoid adsorbed on Alum (for vaccine identification and testing).

PRINCIPLE OF THE TEST

ADI's Diphtheria Toxoid IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Diphtheria Toxoid antigen is bound on the surface of the microtiter strips. Diluted unknowns are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Diphtheria Toxoid antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 15 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured at 450 nm.

Collection and Handling of Samples

Principally serum or plasma (EDTA, heparin) can be used in this test. Serum is separated from whole blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. They can be stored refrigerated (4-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. Unknowns should not be frozen and thawed repeatedly. Lipemia, hemolysis or bacterial contamination can cause inaccurate results. All unknowns must be diluted 1:100 with ready-to-use diluent (e.g. 5 µL serum + 500 µL diluent). No other components of the kit require dilution.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (25-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

TMB (substrate), H2SO4 (stop solution)
All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

REAGENT PREPRATION

Dilute wash buffer 1 + 19; eg. 10 mL Washing Buffer + 190 mL distilled water. Store at 4°C.

QUALITY CONTROL

Each laboratory should utilize controls at several levels to monitor assay performance. The controls should be treated as unknown. Values obtained should be in a agreement with the assigned values of the control. Controls can be obtained from commercially available sources but should not contain sodium azide as preservative.

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.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).* **Dilute wash buffer (1:19) with distilled water .**

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. Label or mark the microtiter well strips to be used on the plate.

1. Pipet **100 µl of ready-to-use standards and diluted samples (1:100)** into appropriate wells in *duplicate*. Leave one well for substrate blank. Cover the plate and incubate for **60 minutes** at 37°C..

2. Aspirate and **wash the wells 3 times with 300 µl wash buffer**. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add **100 µl of enzyme conjugate** into all wells except for the substrate blank well**.** Mix gently for 5- 10 seconds. Cover the plate and incubate for **30 minutes** at room temperature (20-25°C).

4. Aspirate and **wash the wells 3 times with 300 µl wash buffer** as in step 2.

5. Dispense **100 µl TMB substrate into all wells**. Mix gently for 5-10 seconds.

6. Cover the plate and incubate for **15 minutes** at room temperature (20-25°C) in the dark. Blue color develops in positive wells.

7. Stop the reaction by adding **100 µl** of stop solution to all wells. Mix gently for 5-10 seconds. Blue color turns yellow. Read the plate at 450 nm within 30 min.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. do not touch the bottom of the wells.

Limitations, Précautions.

- For *in-vitro* diagnostic 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.
- 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, and they should not be mixed with one another.
- All reagents have to be used within the expiry period.