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

930-100-TTH Human Anti-Tetanus Toxin/Toxoid IgG ELISA kit

930-120-TMA Mouse Anti-Tetanus Toxin/Toxoid IgA ELISA kit

930-130-TMG Mouse Anti-Tetanus Toxin/Toxoid IgG ELISA kit

930-140-TMM Mouse Anti-Tetanus Toxin/Toxoid IgM ELISA kit

930-210-TRG Rabbit Anti-Tetanus Toxin/Toxoid IgG ELISA kit

930-220-TRM Rabbit Anti-Tetanus Toxin/Toxoid IgM ELISA kit

930-310-TGG G. pig Anti-Tetanus Toxin/Toxoid IgG ELISA kit

930-320-TGM G. pig Anti-Tetanus Toxin/Toxoid IgM ELISA kit

930-410-TKG Monkey Anti-Tetanus Toxin/Toxoid IgG ELISA kit

VAC-TTX-310 VacciGel Direct ELISA for the measurement of **Tetanus Toxoid** in Vaccines formulated in Alum, 96 tests

VAC-TTX-310 Tetanus Toxoid/Toxin (TTX) ELISA for the measurement **TTX** in biological buffer

VAC-DTX-200 VacciGel Direct ELISA for the measurement of **Diphtheria Toxoid** in Vaccines formulated in Alum, 96 tests

VAC-DTX-210 Diphtheria Toxoid/Toxin (DTX) ELISA for the measurement **DTX** in biological buffer

VAC-HBS-100 VacciGel Direct ELISA for the measurement of **Hepatitis B Vaccine** (HBsAg) formulated in Alum, 96 tests

VAC-HCG-500 VacciGel Direct ELISA for the measurement of **HCG** (contamination) in Vaccines formulated in Alum, 96 tests

VAC-PTX-400 VacciGel Direct ELISA for the measurement of **Pertussis Toxoid** in Vaccines formulated in Alum, 96 tests

VAC-PTX-410, Pertussis Toxoid/Toxin (PTX) ELISA for the measurement **PTX** in biological buffer

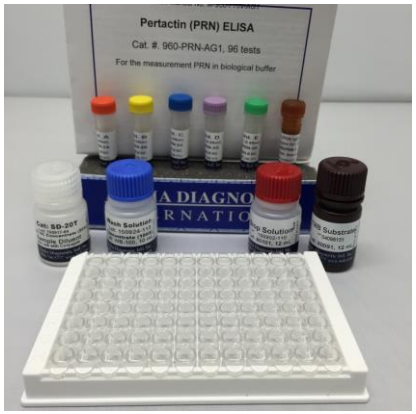
Instruction Manual No. M-960-PRN-AG1

Pertactin (PRN) ELISA

Cat. #. 960-PRN-AG1, 96 tests

For the measurement PRN in biological buffer

For In Vitro Research Use Only (RUO)



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Pertactin (PRN) ELISA for the measurement PRN in biological buffer, 96 tests

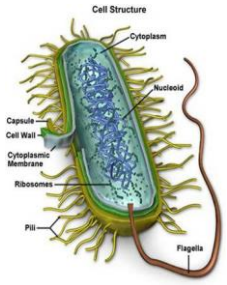
Kit Components, 96 tests	Cat #
Anti-PRN coated Strip plate (8x12 wells)	960PRN-1
PRN **Std. A** (12.5 ng/ml), 0.65 ml	960PRN-2A
PRN **Std. B** (25 ng/ml), 0.65 ml	960PRN-2B
PRN **Std. C** (50 ng/ml), 0.65 ml	960PRN-2C
PRN **Std. D** (100 ng/ml), 0.65 ml	960PRN-2D
PRN **Std. E** (200 ng/ml), 0.65 ml	960PRN-2E
Standards are provided in a stabilizing buffer containing 0.1% Proclin-300	
Sample/Conjugate Diluent (20X), solution, 10 ml	SD20-T
Anti-PRN IgG-HRP Conjugate,**0.25 ml (50X),** Dilute 1:50 with Conj. Diluent	960PRN-3
Wash buffer (100X), 10 ml; dilute 1:100 with water	W B - 1 0 0
TMB substrate, Solution, 12 ml	80091
Stop solution, 1 2 m l	8 0 1 0 1
Instruction Manual #M-960-PRN-AG1	1

Intended Use

Pertactin (PRN) ELISA is a sandwich ELISA Kit suitable for detecting and measuring B. pertussis pertactin (protein) in vaccines or biological buffers. It is not suitable for measuring PRN in vaccines that are formulated in Alum. For in vitro research use only (RUO), not for therapeutic or diagnostic use.

General Information

Pertussis, also known as the whooping cough, is a highly contagious disease caused by the bacterium Bordetella pertussis. It derived its name from the "whoop" sound made from the inspiration of air after a cough. Despite generally high coverage with the DTP and DTaP vaccines, pertussis is one of the leading causes of vaccine-preventable deaths world-wide.

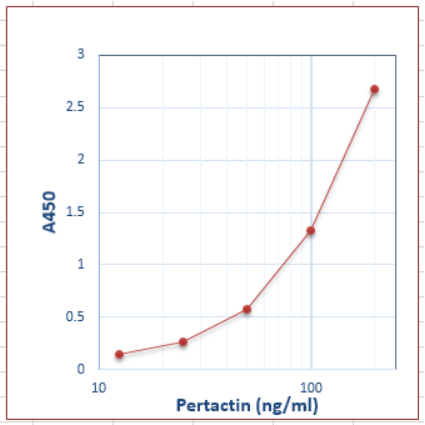


B. pertussis vaccine was first developed in 1920 using whole bacterium. In 1942, the whole-cell pertussis vaccine was combined with diphtheria and tetanus toxoids to generate the first **DTP combination vaccine**. Whole cell vaccines have some side effects. Acellular pertussis vaccines contain between one and five B. pertussis antigens: pertussis toxin (Ptx), filamentous hemagglutinin (FHA), pertactin (Prn), and fimbriae (Fim2 and Fim3). Many aspects of the pathogenesis of pertussis and vaccine correlates of protection are poorly understood. However, antibodies to all components of pertussis antigens (PTX, Prn, FHA and Fim) appear to have a direct correlation with protection. The vaccines with three or more components consisting of filamentous hemagglutinin (FHA), pertussis toxin (PT) and pertactin (PRN) are considered to be more effective than one/two-component pertussis vaccines that contain only PT or both PT and FHA.

Pertactin (PRN or p69 protein) is a highly immunogenic virulence factor of B. Pertussis. Specifically, it is an outer membrane protein that promotes adhesion to tracheal epithelial cells. **Pertussis toxin (PTX or PT**) has numerous biological activities and probably plays

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A_{450} nm	Mean A_{450} nm
A1, A2	**Blanks** (0 ng/ml)	0.34	
B1, B2	**PRN Std. A** (12.5 ng/ml)	0.48	0.14
C1, C2	**PRN Std. B** (25 ng/ml)	0.61	0.27
D1, D2	**PRN Std. C** (50 ng/ml	0.92	0.58
E1, E2	**PRN Std. D** (100 ng/ml)	1.669	1.329
F1, F2	**PRN Std. E** (200 ng /ml	3.02	2.68



N/960-PRN-AG1

A typical std. assay curve (do not use this for calculating sample values). A complete standard curve must be run in every assay to determine sample values

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero or blank (sample diluent only) from the mean absorbance values of standards, control, and samples. Draw the standard curve on a semi-log graph paper by plotting net absorbance values of standards against appropriate PRN concentrations. Read off the PRN concentrations of the control and samples directly from the standard curve. If samples were diluted then the values should be multiplied by the dilution factor.

If ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.

a role in hampering the host immune response. PT is a protein-based A/B-type exotoxin" because they are formed from two subunits. The "A" subunit possesses enzyme activity, and is transferred to the host cell following a conformational change in the membrane-bound transport "B" subunit. **Filamentous hemeagglutinins (FHA)** is one of two hemeagglutinins produced by phase I strains of B. pertussis. **Fimbriae (FIM)** have been considered important vaccine components for many years in both whole-cell and acellular vaccines. B. pertussis expresses two serologically distinct fimbriae composed of either **Fim2** (207-aa; 22.5 kda) or **Fim3** (204-aa, 22 kda) major subunits. Antibody responses to Fim1-3 have been observed in human samples.

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.

PRINCIPLE OF THE TEST

Pertactin (PRN) ELISA kit is based on binding of PRN to an antibody coated on the plate and antibody-HRP conjugate. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of PRN 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 PRN in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader. Table top microfuge

PRECAUTIONS AND SAFETY INSTRUCTIONS

ADI PRN ELISA kit is intended for *in vitro research* use only. The reagents contain proclin-300 (0.1%) as preservative; necessary care should be taken when disposing solutions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

SPECIMEN COLLECTION AND HANDLING

This kit is designed to measure the PRN vaccine formulated in biological buffers (non-alum based vaccines). Do not add azide or other preservatives to vaccines. Presence of vaccine specific buffers, additives must be studies by measuring effects on the standards before measuring samples. This kit is not suitable to measure PRN adsorbed on alum). ADI has other kits to measure PRN in biological buffers.

REAGENTS PREPARATION FOR THE ASSAY

Dilute wash buffer (1:100) with distilled water (10 ml stock in 990 ml). Store at 4oC.

Dilute enzyme conjugate 1:50 (eg; **20 ul of HRP in 980 ul antibody conjugate diluent**). Do not keep working stock of conjugate beyond the assay. Prepare only in required amounts.

STORAGE AND STABILITY

The kit contents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions..

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

All standards, controls, and samples should be tested in duplicate.

1. Dilute samples **with** sample diluent Do not dilute standards. Pipet **100 ul stds** and diluted samples into appropriate wells. Gently mix the plate for 5-seconds by tapping against the palm. Cover the plate and incubate for **60 minutes** at room temperature.

2. **Note:** for ease of loading samples it is recommended that a second **uncoated** microwell plate should be used for sample dilution. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipet.

3. Aspirate and **wash the wells 4 times** with wash buffer (300 ul/well/wash). 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.

4. Pipet **100 ul of diluted Ab-enzyme conjugate** into each well**. Mix gently for 5-10 seconds.** Cover the plate and incubate for **30 minutes** at room temperature.

5. Aspirate and **wash the wells 4 times** with wash buffer(same as in step 4) .

6. Dispense **100 ul TMB substrate solution per well.** Mix gently. Cover the plate and incubate on a plate shaker for **15 minutes** at room temp. incubation time may be + 5 min so as to get maximum A450 =<2.00-3.00). Blue color develops in standards and positive wells.

7. Stop the reaction by adding **100 ul of stop solution** to all wells at the same timed intervals as in step 8. Mix gently for 5-10 seconds to make ensure even color distribution. Blue color turns yellow.

8. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 15 mins after stopping.

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. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

All vaccine samples must be tested at least 2 dilutions that are within the standards range. Samples containing PRN more than highest standards (100 ng/ml) should be diluted further. The results obtained should be multiplied by the appropriate dilution factor.

QUALITY CONTROL

Standards and controls, if available, must perform as stated in the manual.

PERFORMANCE CHARACTERISTICS

DETECTION LIMIT- Based on replicate determinations of the zero standard, the minimum PRN concentration detectable using this assay is ~1 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Specificity

The antibodies used in this kit are specific for B. pertussis toxin or Toxoid with no reactivity with diphtheria or Pertussis or other toxoids. Some preparations of toxoid may have extensive crosslinking of the protein and may impact the detection of the toxoid in this kit. We suggest testing the unmodified and modified toxin.