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User's Manual

Parainfluenza Virus IgA ELISA Kit

REF **DEIA370**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The ELISA Parainfluenza Virus IgA test is a qualitative and quantitative immunoassay for the detection of human antibodies in serum or plasma directed against all relevant human pathogenic Parainfluenza Viruses.

General Description

Parainfluenza Viruses cause mild to severe upper and lower respiratory tract infections. Parainfluenza Viruses are surpassed only by Respiratory Syncytial Virus as the cause of severe lower respiratory tract infections in children. Infections are transmitted by droplet infection or direct contact via mucous membranes of the eyes, mouth, or nose. Currently, Parainfluenza Viruses can be divided into four type of which types 1-3 are most frequently detected.

Principles of Testing

The ELISA (Enzyme-Linked Immunosorbent Assay) is an immunoassay suited to the detection of antibodies. The reaction is based on the specific interaction of antibodies with their corresponding antigen. The test strips of the SERION ELISA classic microtiter plate are coated with specific antigens of the pathogen of interest. If antibody in a sample is present, they bind to the fixed antigen. A secondary antibody, which has been conjugated with the enzyme alkaline phosphatase, detects and binds to the antigen-antibody complex. The colorless substrate pnitrophenylphosphate is then converted into the colored product p-nitrophenol. The signal intensity of this reaction product is proportional to the concentration of the antibody in the sample and is measured photometrically.

Reagents And Materials Provided

1. Break apart microtiter test strips each with eight antigen coated single wells, (altogether 96), 1 frame. The coating material is inactivated. 12 pieces

2. Standard serum (ready-to-use), Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Amaranth O. 2 × 2 ml.

3. Negative control serum (ready-to-use), Human serum in protein-containing phosphate buffer; negative for anti-HIV Ab, HBs-Ag (Hepatitis B-Virus surface Antigen) and anti-HCV Ab; Preservative: <0.1% sodium azide; coloring: Lissamin Green V. 2 ml

4. Anti-human IgA conjugate (ready-to-use), Anti-human IgA polyclonal antibody, Conjugated to alkaline phosphatase, stabilized with protein stabilization solution; Preservative: <0.1% methylisothiazolone, <0.1% bromnitrodioxane. 13ml

5. Washing solution concentrate (sufficient for 1000ml), Sodium chloride solution with Tween 20 and 30mM Tris-HCl, pH 7.4; Preservative: <0.1% sodium azide. 33.3ml

6. Dilution buffer (ready-to-use), Protein-containing phosphate buffer with Tween 20; Preservative: <0.1% sodium azide; coloring: 0.01g/l Bromphenol blue. 2 × 50ml

7. Stopping solution (ready-to-use), <0.1N sodium hydroxide, 40mM EDTA. 15ml

8. Substrate (ready-to-use), Para-nitrophenylphosphate in solvent-free buffer; Preservative: <0.1% sodium azide. 13ml

Materials Required But Not Supplied

1. Common laboratory equipment
2. Photometer for microtiter plates with filter, wavelength 405nm, recommended reference wavelength 620nm-690nm (e.g., 650nm)
3. Microtiter plate washer
4. Incubator 37°C
5. Moist chamber
6. Distilled water
7. Optional: ELISA control

Storage

Reagent	Storage	Stability
Microtiter strips (coated with antigen)	Unopened After opening at 2-8°C in closed aluminum bag with desiccant	See expiry date Minimum shelf-life: four weeks
Control sera / Standard sera	Unopened / after opening at 2-8°C	See expiry date
Conjugate	Unopened / after opening at 2-8°C	See expiry date
Dilution buffer	Unopened / after opening at 2-8°C	See expiry date
Washing solution	Unopened / after opening at 2-8°C Working dilution at 2-8°C Working dilution at room temperature	See expiry date 2 weeks 1 week Substrate: Unopened / after opening at 2-8°C Stopping solution: Unopened / after opening at 2-8°C

Specimen Collection And Preparation

Lipaemic, hemolytic or icteric samples (serum or plasma) should only be tested with caution. Obviously contaminated samples should not be tested. Serum or plasma (EDTA, citrate, heparin) collected according to standard laboratory methods are suitable samples. Samples must not be thermally inactivated.

1. Dilution of Samples

Before running the test, samples (V_1) must be diluted in dilution buffer (V_2) as follows:

$V_1 + V_2 = 1:400$	add	10ul	sample
	each to	1000ul	dilution buffer (= 1:100)
		50ul	from the first dilution step
	each to	200ul	dilution buffer (= 1:4)

After dilution and before pipetting into the microtiter plate, the samples must be mixed thoroughly to prepare a homogenous solution.

2. Sample Storage

Samples should not be stored for more than 7 days at 2-8°C. Extended storage is possible at ≤-20°C. Avoid repeated freezing and thawing of samples. Diluted samples can be stored at 2-8°C for one week.

Reagent Preparation

Bring all reagents to room temperature before testing.

1. Microtiter Test Strips

The microtiter test strips labeled with abbreviations for pathogen and immunoglobulin class are packed with a desiccant in an aluminum bag. To open the aluminum bag of the microtiter plate, please cut off the top of the marked side only in order to guarantee proper resealing. Take unrequired wells out of the frame and put them back into the aluminum bag. Close bag carefully to ensure airtight conditions. Do not use strips if the aluminum bag is damaged or if the bag with remaining strips and desiccant was not properly resealed.

2. Control Sera / Standard Sera (ready-to-use)

Control and standard sera are ready-to-use and must not be diluted any further. For each test run (independent of the number of microtiter test strips to be used) control and standard sera must be included. Standard and cut-off sera should be set up in duplicate. Do not treat control sera with Rf-absorbent.

3. Anti-human IgA AP-Conjugate (ready-to-use)

The required conjugate concentration (i.e., +, ++, +++) is indicated on the quality control certificate. Please refer also to the specification on the label.

4. Washing Solution (Concentrate)

Dilute washing buffer concentrate (V_1) 1:30 with distilled H_2O to a final volume of V_2.

Example:

buffer concentrate (V_1)	final volume (V_2)
33.3 ml	1000 ml
1 ml	30 ml

5. Dilution Buffer for Samples (ready-to-use)

6. Substrate (ready-to-use)

Substrate in unopened bottle may have a slight yellow color which does not reduce the quality of the product!

7. Stopping Solution (ready-to-use)

Assay Procedure

1. Place the required number of wells in the frame and prepare a protocol sheet.
2. Add each 100ul of diluted sample or ready-to-use controls into the appropriate wells of microtiter test strips. Spare one well for substrate blank, e.g.:

   A1 Substrate blank

   B1 Negative control

   C1 Standard serum

   D1 Standard serum

   E1 Sample 1 . . .

   F1 Sample 2 . . .
3. Sample incubation for 60 minutes (+/- 5 min) at 37°C (+/- 1°C) in moist chamber.
4. After incubation wash all wells with washing solution (by automated washer or manually):

   aspirate or shake out the incubation solution

   fill each well with 300ul washing solution

   aspirate or shake out the washing solution

   repeat the washing procedure 3 times (altogether 4 times!)

   dry by tapping the microtiter plate on a paper towel
5. Addition of conjugate: Add 100ul of the ready-to-use IgA conjugate to the appropriate wells (except substrate blank).
6. Conjugate incubation for 30 minues (+/- 1 min) at 37°C (+/- 1°C) in moist chamber.
7. After incubation wash all wells with washing solution (see above).
8. Addition of substrate: Add 100ul of ready-to-use substrate solution to each well (including well for substrate blank!)
9. Substrate incubation for 30 minutes (+/- 1 min) at 37°C (+/- 1°C) in moist chamber.
10. Stopping the reaction: Add 100ul of stopping solution to each well, shake microtiter plate gently to mix.
11. Read extinction: Read optical density (OD) within 60 minutes at 405nm against substrate blank, reference wavelength between 620nm and 690nm (e.g. 650nm).

Quality Control

For the periodic verification of the test method, in order to fulfill the requirements of laboratory internal quality management systems, we recommend using ELISA controls to determine precision and accuracy of ELISA

test runs. The use of ELISA controls is described in specific instruction manuals.

Calculation

1. ELISA Parainfluenza Virus IgA

The mathematical curve fitting for antibody quantification with SERION ELISA classic immunoassays is based on the 4-parameter logistic (4 PL) function.

$$Activity\ (U/ml) = e^{C-\frac{1}{B}\ln\left(\frac{D-A}{OD(Patient)*F-A}-1\right)}$$

The 4 parameters A, B, C, and D are representative for the exact shape of the standard curve:

Parameter A: Lower asymptote (OD)

Parameter B: Slope of the curve

Parameter C: Inflection point

Parameter D: Upper asymptote (OD)

CD establishes a lot-specific 4 PL standard curve for this ELISA immunoassay in multiple test runs under optimal test conditions. The four parameters are indicated on the quality control certificate of each individual ELISA test.

For the adaptation of the test level to the given 4 PL standard curve, the correction factor F is calculated by dividing the standard reference OD value indicated on the quality control certificate with the measured, and consequently test run-specific, standard OD value.

$$F = \frac{STD\ reference\ OD\ value}{measured\ \ STD\ OD\ value}$$

By multiplying the OD values obtained from samples with the correction factor F, the level of each individual test run is adjusted to the given 4 PL standard curve. Thereby, interassay deviations are compensated for and antibody activities can be directly evaluated from the 4 PL standard curve.

After subtraction of the substrate blank from all measured OD values and calculation of the mean OD value of the standard serum (STD), tested in duplicate, a range of possibilities are available for the evaluation of antibody activities from the optical measurement signals (OD) of samples. They are described in separate manuals.

2. Borderline Ranges

The borderline ranges of the ELISA Parainfluenza Virus IgA test are specified on the quality control certificates and indicate the range of borderline test results. Values below this range indicate a negative result; values above the borderline range indicate a positive result.

3. Limits of Quantification

The limits of quantification are specified on the quality control certificate of the ELISA Parainfluenza Virus IgA. The linearity of dilution within this range has been demonstrated in comprehensive evaluation studies. If

a sample shows a test result above the upper limit of quantification, the sample may be tested at a higher dilution. The resulting antibody activity must then be multiplied by the additional dilution factor.

4. Criteria of Validity

a. The substrate blank must be <0.25 OD.

b. The negative control must be negative.

c. By use of quantitative ELISA tests, the mean OD value (after subtraction of the substrate blank!) of the standard serum must be within the validity range which is given on the lot-specific quality control certificate.

d. By use of qualitative SERION ELISA classic tests, the OD value of the positive control and the mean OD value of the cut-off serum must be within the validity ranges which are given on the lot-specific quality control certificate of the kit (after subtraction of the substrate blank!).

e. The variation of OD values of the standard serum or cut-off serum must not be higher than 20%.

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

Precautions

Optimum results can only be achieved if the instructions are strictly followed. Only use ELISA reagents when using ELISA immunoassays. The components must not be exchanged for reagents of other manufacturers. Standard and control sera of ELISA immunoassays are defined exclusively for the test kit to be used and must not be used in other lots.

Each ELISA test contains a ready-to-use sample dilution buffer. In some cases the use of special dilution buffers is necessary to guarantee consistent quality and reliable results. The dilution buffers can be used irrespective of the lots.

There are three different conjugate concentrations for each immunoglobulin class (IgA, IgG, IgM) indicated on the label as + (low), ++ (medium), and +++ (high). Conjugates with the same concentration and of the same immunoglobulin class are interchangeable and can be used for other ELISA immunoassays irrespective of the lot and the test. Dilution or alteration of the reagents may result in a loss of sensitivity. Use aseptic techniques when removing aliquots from the reagent tubes to avoid contamination.

The ELISA is designed for use by qualified personnel who are familiar with good laboratory practice. All kit reagents and human samples should be handled carefully using established good laboratory practice.

1. This kit contains human blood components. Although all control- and cut-off sera have been tested and found negative for anti-HIV Ab, HBs-Ag (Hepatitis B Virus surface Antigen) and anti-HCV Ab, they should be considered potentially infectious.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas in which samples or kit reagents are handled.
4. Wear disposable gloves, laboratory coat, and safety glasses while handling kit reagents or samples. Wash hands thoroughly afterwards.
5. Samples and other potentially infectious material should be decontaminated after use.
6. Reagents should be stored safely and be inaccessible to unauthorized access, e.g. children.