Version 06-05/24

User's Manual

Human Influenza A IgA ELISA Kit

REF **DEIA2368**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The ELISA test kit provides a semiquantitative in vitro assay for human antibodies of the IgA class against Influenza A virus in serum or plasma.

Principles of Testing

The test kit contains microtiter strips each with 8 break-off reagent wells coated with Influenza A virus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgA antibodies (also IgG and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgA (enzyme conjugate) catalysing a colour reaction.

Reagents And Materials Provided

1. Microplate wells, coated with antigens, 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use --- 12 x 8 **.STRIPS.**
2. Calibrator (IgA, human), ready for use, dark red, 1 x 2.0 ml **.CAL.**
3. Positive control (IgA, human), ready for use, blue, 1 x 2.0 ml **.POS CONTROL.**
4. Negative control (IgA, human), ready for use, green 1 x 2.0 ml **.NEG CONTROL.**
5. Enzyme conjugate, peroxidase-labelled anti-human IgA (rabbit), ready for use, orange, 1 x 12 ml **.CONJUGATE.**
6. Sample buffer, ready for use, light blue, 1 x 100 ml **.SAMPLE BUFFER.**
7. Wash buffer, 10x concentrate, colourless, 1 x 100 ml **.WASH BUFFER 10x.**
8. Chromogen/substrate solution, TMB/H_2O_2, ready for use, colourless, 1 x 12 ml **.SUBSTRATE.**
9. Stop solution, 0.5 M sulphuric acid, ready for use, colourless, 1 x 12 ml **.STOP SOLUTION.**
10. Test instruction --- 1 booklet
11. Quality control certificate --- 1 protocol

Storage

The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Specimen Collection And Preparation

Sample material: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** sample buffer. For example: dilute 10 µl serum in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: The Calibrator and controls are prediluted and ready for use, do not dilute them.

Plate Preparation

STD.(pg/ml)	OD-1	OD-2	Average	Corrected
0	0.072	0.074	0.073	0.000
15.625	0.099	0.101	0.1	0.027
31.25	0.125	0.129	0.127	0.054
62.5	0.21	0.216	0.213	0.14
125	0.361	0.371	0.366	0.293
250	0.726	0.748	0.737	0.664
500	1.421	1.463	1.442	1.369
1000	2.373	2.441	2.407	2.334

The pipetting protocol for microtiter strips is an example for the **semiquantitative analysis** of 24 patient sample (P 1 to P 24).

The calibrator (C), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.

The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.

Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Reagent Preparation

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- Coated wells: Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).

Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- Calibrator and controls: Ready for use. The reagents must be mixed thoroughly before use.

- Enzyme conjugate: Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- Sample buffer: Ready for use.

- Wash buffer: The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to +37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).

For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.

The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- Chromogen/substrate solution: Ready for use. Close the bottle immediately after use, as the contents are sensitive to light . The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- Stop solution: Ready for use.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The control sera used have been tested negative for HBsAg, anti-HCV, anti-HIV-1 and antiHIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid skin contact.

Assay Procedure

Sample incubation: (1st step)

Transfer 100 µl of the calibrator, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for 30 minutes at room temperature (+18°C to +25°C).

Washing:

Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.

Automatic: Wash the reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Mode").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing, thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (>10 µl) in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values. Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)

Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgA) into each of the microplate wells. Incubate for 30 minutes at room temperature (+18°C to +25°C).

Washing:

Empty the wells. Wash as described above.

Substrate incubation: (3rd step)

Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for 15 minutes at room temperature (+18°C to +25°C) (protect from direct sunlight).

Stopping:

Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity should be made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Calculation

The extinction value of the calibrator defines the upper limit of the reference range of non-infected persons (cut-off) recommended by Creative-Diagnostics. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

Extinction of the control or patient sample / Extinction of calibrator = Ratio

Creative-Diagnostics recommends interpreting results as follows:

Ratio < 0.8: Negative

Ratio ≥ 0.8 to <1.1: borderline

Ratio ≥ 1.1: positive

In cases of borderline test results, an additional patient sample should be taken 7 days later and retested in parallel with the first patient sample. The results of both samples allow proper evaluation of titer changes.

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another the sample should be retested.

For diagnosis, the clinical symptoms of the patient should always be taken into account along with the serological results.

Reference Values

The levels of the anti-influenza A virus antibodies (IgA) were analyzed with this ELISA in a panel of 500 healthy blood donors. With a cut-off ratio of 1.0, 29.8% of the blood donors were anti-influenza A virus positive (IgA).

Performance Characteristics

Calibration: As no international reference serum exists for antibodies against influenza A viruses, results are provided in the form of ratios which are a relative measure for the concentration of antibodies.

For every group of tests performed, the extinction values of the calibrator and the ratios of the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the

test results may be inaccurate and the test should be repeated.

The activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature during substrate incubation, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

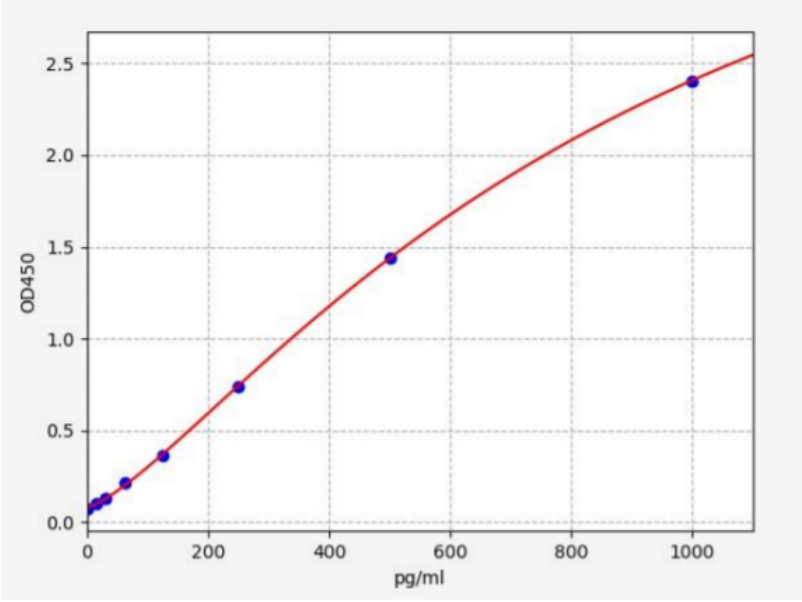
Antigen: The antigen source is provided by inactivated allantois fluid of chicken embryos infected with the "Texas" (H3N2) strain of Influenza A virus, „Singapore" (H1N1) strain of Influenza A virus and "California" (H1N1, Porcine Influenza).

Detection Limit

The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The detection limit of the Anti-Influenza A Virus ELISA (IgA) is ratio 0.04.

Specificity

This ELISA showed cross reactivity with antibodies against influenza B virus.



Reproducibility

The reproducibility of the test was investigated by determining the intra- and interassay coefficients of variation using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	86-102	97
EDTA Plasma(n=5)	86-99	93
Heparin Plasma(n=5)	87-100	94

Interferences

Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.