Version 22-04/24

User's Manual

Human Papilloma Virus IgG ELISA kit

REF **DEIA-F678S**

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

Enzyme Immuno Assay (EIA) for determination of IgG class antibodies to Human Papilloma Virus (or HPV) in human plasma and sera.

The product is supplied for research purpose only. It is not for use in the diagnosis or for the follow-up of patients administered with the vaccines containing HPV antigens.

Principles of Testing

Microplates are coated with recombinant VLP's derived from HPV Type 6, 11, 16E, 18. In the 1st incubation, the solid phase is treated with diluted samples and anti-HPV IgG are captured, if present, by the antigens.

After washing out all the other components of the sample, in the 2nd incubation bound anti-HPV IgG are detected by the addition of anti hIgG antibody, labeled with peroxidase (HRP).

The enzyme captured on the solid phase, acting on the substrate/chromogen mixture, generates an optical signal that is proportional to the amount of anti-HPV IgG antibodies present in the sample. A cut-off value turns the measured optical densities into positive or negative results.

Reagents And Materials Provided

1. Microplate: 12 strips × 8 breakable microwells coated with recombinant VLP's derived from HPV Type 6, 11, 16 E, 18. Plates are sealed into a bag with desiccant. Allow the microplate to reach room temperature before opening; reseal unused strips in the bag with desiccant and store at 4°C.

2. Negative Control: 1×4.0 mL/vial. Ready to use and pale yellow color coded. Contains human serum negative for IgG anti HPV, 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

3. Positive Control: 1×4.0 mL/vial. Ready to use and dark green color coded. Contains human serum positive for IgG anti HPV, 2% casein, 10 mM Na-citrate buffer pH 6.0 ± 0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives.

4. 20×Wash buffer concentrate: 1×60 mL/bottle. 20× concentrated solution. Once diluted, the wash solution contains 10 mM phosphate buffer pH 7.0+/-0.2, 0.05% Tween 20 and 0.05% Kathon GC.

5. Enzyme Conjugate: 1×16 mL/vial. Ready to use and red colour coded. It contains Horseradish peroxidase conjugated polyclonal antibodies to human IgG, 5% BSA, 10 mM Tris buffer pH 6.8+/-0.1, 0.1% Kathon GC and 0.02% gentamicine sulphate as preservatives.

6. Chromogen/Substrate: 1×16 mL/vial. It contains 50 mM citrate-phosphate buffer pH 3.5-3.8, 4% dimethylsulphoxide, 0.03% tetra-methylbenzidine (or TMB) and 0.02% hydrogen peroxide (or H_2O_2).

Note: To be stored protected from light as sensitive to strong illumination.

7. Sulphuric Acid: H_2SO_4 0.3M, 1×15 mL/vial. It contains 0.3 M H_2SO_4 solution.

8. Specimen Diluent: 2×60mL/vial. It contains 2% casein, 10 mM Na-citrate buffer pH 6.0 +/-0.1, 0.1% Tween 20, 0.09% Na-azide and 0.1% Kathon GC as preservatives. To be used to dilute the sample.

9. Plate sealing foils 2

10. Package insert 1

Materials Required But Not Supplied

1. Calibrated Micropipettes (1000, 100 and 10 µL and disposable plastic tips.
2. EIA grade water (bidistilled or deionised, charcoal treated to remove oxidizing chemicals used as disinfectants).
3. Timer with 60 minute range or higher.
4. Absorbent paper tissues.
5. Calibrated ELISA microplate thermostatic incubator (dry or wet) set at +37°C (± 0.5°C tolerance).
6. Calibrated ELISA microwell reader with 450nm (reading) and possibly with 620 - 630nm (blanking) filters.
7. Calibrated ELISA microplate washer.
8. Vortex or similar mixing tools.

Storage

2°C to 8°C

Specimen Collection And Preparation

1. Blood is drawn aseptically by venipuncture and plasma or serum is prepared using standard techniques of preparation of samples for clinical laboratory analysis. No influence has been observed in the preparation of the sample with citrate, EDTA and heparin.
2. Samples have to be clearly identified with codes or names in order to avoid misinterpretation of results. Bar code labeling and electronic reading is strongly recommended.
3. Haemolysed ("red") and visibly hyperlipemic ("milky") samples have to be discarded as they could generate false results. Samples containing residues of fibrin or heavy particles or microbial filaments and bodies should be discarded as they could give rise to false results.
4. Sera and plasma can be stored at 2°C-8°C for up to five days after collection. For longer storage periods, samples can be stored frozen at –20°C for several months. Any frozen samples should not be frozen/thawed more than once as this may generate particles that could affect the test result.
5. If particles are present, centrifuge at 2000 rpm for 20 min or filter using 0.2-0.8 µm filters to clean up the sample for testing.

Plate Preparation

Allow the microplate to reach room temperature (about 1 hr) before opening the container. Check that the desiccant is not turned to dark green, indicating a defect of storage. Unused strips have to be placed back into the aluminium pouch, in presence of desiccant supplied, firmly zipped and stored at 2°C - 8°C. When opened the first time, residual strips are stable till the indicator of humidity inside the desiccant bag turns from yellow to green.

Reagent Preparation

1. Controls:

Ready to use. Mix well on vortex before use.

2. Wash buffer concentrate:

The whole content of the concentrated solution has to be diluted 20x with bidistilled water and mixed gently end-over-end before use. During preparation avoid foaming as the presence of bubbles could impact on the efficiency of the washing cycles. **Note: Once diluted, the wash solution is stable for 1 week at 2°C-8°C.**

3. Enzyme Conjugate:

Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong illumination, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, possible sterile disposable container

4. Chromogen/Substrate:

Ready to use. Mix well on vortex before use. Be careful not to contaminate the liquid with oxidizing chemicals, air-driven dust or microbes. Do not expose to strong illumination, oxidizing agents and metallic surfaces. If this component has to be transferred use only plastic, possible sterile disposable container.

5. Sample Diluent

Ready to use component. Mix carefully on vortex before use.

6. Sulphuric Acid:

Ready to use. Mix well on vortex before use.

Legenda: R 36/38 = Irritating to eyes and skin.

S 2/26/30 = In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

Assay Procedure

Pre-Assay Controls And Operations

1. Check the expiration date of the kit printed on the external label (primary container). Do not use if expired.
2. Check that the liquid components are not contaminated by visible particles or aggregates.
3. Check that the Chromogen (TMB) is colourless or pale blue by aspirating a small volume of it with a sterile plastic pipette.
4. Check that no breakage occurred in transportation and no spillage of liquid is present inside the box (primary container). Check that the aluminium pouch, containing the microplate, is not punctured or damaged.
5. Dilute all the content of the 20x concentrated Wash Solution as described above.
6. Allow all the other components to reach room temperature (about 1 hr) and then mix gently on vortex all liquid reagents.
7. Set the ELISA incubator at +37°C and prepare the ELISA washer by priming with the diluted washing solution, according to the manufacturer's instructions. Set the right number of washing cycles as found in the validation of the instrument for its use with the kit.

8. Check that the ELISA reader is turned on or ensure it will be turned on at least 20 minutes before reading.
9. If using an automated work station, turn on, check settings and be sure to use the right assay protocol.
10. Check that the micropipettes are set to the required volume.
11. Check that all the other equipment is available and ready to use.
12. In case of problems, do not proceed further with the test and advise the supervisor.

Procedure

The assay has to be carried out according to what reported below, taking care to maintain the same incubation time for all the samples in testing.

1. Dilute samples 1:101 into a properly defined dilution tube (example: 1000 µL Sample Diluent + 10 µL sample). Do not dilute the Controls as they are ready to use. Mix carefully all the liquid components on vortex and then proceed as described below.
2. Place the required number of Microwells in the microwell holder. Leave A1 well empty for the operation of blanking.
3. Dispense 100 µL of Negative Control and of Positive Control in duplicate. Then dispense 100 µL of diluted samples in each properly identified well.
4. Incubate the microplate for 60 min at +37°C. Important note: Strips have to be sealed with the adhesive sealing foil, supplied, only when the test is carried out manually. Do not cover strips when using ELISA automatic instruments.
5. Wash the microplate with an automatic washer as reported previously.
6. Pipette 100 µL Enzyme Conjugate into each well, except the A1 well, and cover with the sealer. Check that this red coloured component has been dispensed in all the wells, except A1. Important note: Be careful not to touch the plastic inner surface of the well with the tip filled with the Enzyme Conjugate. Contamination might occur.
7. Incubate the microplate for 60 min at +37°C.
8. Wash microwells as in step 5.
9. Pipette 100 µL Chromogen/Substrate mixture into each well, the blank well included. Then incubate the microplate at room temperature (18-24°C) for 20 minutes. Important note: Do not expose to strong direct illumination. High background might be generated.
10. Pipette 100 µL Sulphuric Acid into all the wells using the same pipetting sequence as in step 9. Addition of acid will turn the positive control and the positive samples from blue to yellow.
11. Measure the colour intensity of the solution in each well, at 450 nm filter (reading) and possiblyat 620-630 nm (background subtraction), blanking the instrument on A1.

General Important notes:

1. If the second filter is not available ensure that no finger prints are present on the bottom of the microwell before reading at 450 nm. Finger prints could generate false positive results on reading.
2. Reading has to be carried out just after the addition of the Stop Solution and anyway not any longer than 20 minutes after its addition. Some self oxidation of the chromogen can occur leading to high background.

Quality Control

A validation check is carried out on the controls and the calibrator any time the kit is used in order to venty

whether the performances of the assay are as expected and required by the IVDD directive 9e/79/EC.

Control that the following dats are matched:

Blank well A1: < 0.100 OD450 nm

Negative Control: < 0.150 OD450 nm after blanking

Positve Control: > 0.500 OD 450 nm after blanking

If the results of the test match the requirements stated above, proceed to the next section. If they do not, do not proceed any further and operate as follows:

Blank well >0.100 OD450 nm:

1. that the Chrormogen/Substrate solution has not got contaminated during the assay

Negative Control> 0.150 OD450 nm after blanking

1. that the washing procedure and the washer settings are as validated in the pre qualificaton study.
2. that the proper washing solution has been used and the washer has been primed with It before use;
3. that no mistake has been done in the assay procedure (dispensaton of a positive calibrator instead of the negative one;
4. that no contamination of the negatve calibrator or of their wells has occurred due spills of positive samples or the enzyme conjugate;
5. that micropipetes haven't got contaminated with positive samples or with the enzyme conjugate.
6. that the washer needles are not blocked or partially obstructed.

Positive Control <0.500 OD450 nm

1. that the procedure has been correctly executed;
2. that no mistake has been done in its drstribution (dispensation of a wrong calibrator instead);
3. that the washing procedure and the washer settings are as validated in the pre qualification study,
4. that no extemal contamination of the positive control has occurred.

Calculation

If data are valid, calculate the mean OD 450 nm value of the Negative Control (or NC) and then apply the following formulation to calculate the cut-off value:

NC + 0.250 = Cut-Off

Important Note: When the calculation of results is made by an automatic work station, assure that the system has been loaded with the right formulation.

Interpretation Of Results

Samples with an OD 450 nm / 620-630 nm lower that the Cut-Off value are considered not reactive for lgG specifc to the HPV antigens present in the vaccine.

Samples with an OD 450 nm /620-630 nm higher than the Cut-Off value are considered positive for lgG specific to the HPV antigens present in the vaccine.

In case the quantification of IgG present in positive samples s required to better monitor the immunological response to the vaccine in a prolonged time, caiculate for each sample the value.

OD Sample/Cut-Off(or S/Co)

that provide an index whose value is directly proportional to the content of IgG in the sample.

Precautions

For Research Use Only. Not for in vitro diagnostic use.

1. Micropipettes have to be calibrated to deliver the correct volume required by the assay and must be submitted to regular decontamination (household alcohol, 10% solution of bleach, hospital grade disinfectants) of those parts that could accidentally come in contact with the sample. They should also be regularly maintained in order to show a precision of 1% and a trueness of +/-2%. Decontamination of spills or residues of kit components should also be carried out regularly.
2. The ELISA incubator has to be set at +37°C (tolerance of +/-0.5°C) and regularly checked to ensure the correct temperature is maintained. Both dry incubators and water baths are suitable for the incubations, provided that the instrument is validated for the incubation of ELISA tests.
3. The ELISA washer is extremely important to the overall performances of the assay. The washer must be carefully validated and correctly optimised using the kit controls and reference panels, before using the kit for routine laboratory tests. Usually 4-5 washing cycles (aspiration + dispensation of 350 µL/well of washing solution = 1 cycle) are sufficient to ensure that the assay performs as expected. A soaking time of 20-30 seconds between cycles is suggested. In order to set correctly their number, it is recommended to run an assay with the kit controls and well characterized negative and positive reference samples, and check to match the values reported below in the section O "Internal quality Control". Regular calibration of the volumes delivered by, and maintenance (decontamination and cleaning of needles) of the washer has to be carried out according to the instructions of the manufacturer.
4. Incubation times have a tolerance of +5%.
5. The ELISA microplate reader has to be equipped with a reading filter of 450nm and ideally with a second filter (620-630 nm) for blanking purposes. Its standard performances should be (a) bandwidth < 10 nm; (b) absorbance range from 0 to > 2.0; (c) linearity to > 2.0; repeatability > 1%. Blanking is carried out on the well identified in the section "Assay Procedure". The optical system of the reader has to be calibrated regularly to ensure that the correct optical density is measured. It should be regularly maintained according to the manufacturer's instructions. When using an ELISA automated work station, all critical steps (dispensation, incubation, washing, reading, data handling) have to be carefully set, calibrated, controlled and regularly serviced in order to match the values reported in the section "Internal quality Control". The assay protocol has to be installed in the operating system of the unit and validated as for the washer and the reader. In addition, the liquid handling part of the station (dispensation and washing) has to be validated and correctly set. Particular attention must be paid to avoid carry over by the needles used for dispensing and for washing. This must be studied and controlled to minimize the possibility of contamination of adjacent wells. The use of ELISA automated work stations is recommended when the number of samples to be tested exceed 20-30 units per run.