



User's Manual

CVPx™ Human HPV 16 L1-VLP IgG ELISA Kit



DEIA-JY2493



96T



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

For the qualitative determination of human papillomavirus type 16 L1-VLPs (HPV16 L1) antibody (IgG) concentrations in human serum, plasma.

Principles of Testing

This assay employs the qualitative enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with antigen. Samples are pipetted into the wells with anti-human IgG conjugated Horseradish Peroxidase (HRP). Any antibodies specific for the antigen present will bind to the pre-coated antigen. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human HPV 16 L1 antibody (IgG) bound in the initial step. The color development is stopped and the intensity of the color is measured.

Reagents And Materials Provided

1. Coated assay plate: 1(96 wells)
2. Negative Control: 1 x 800 µl
3. Positive Control: 1 x 800 µl
4. HRP-conjugate (100 x concentrate): 1 x 120 µl
5. HRP-conjugate Diluent: 1 x 12 ml
6. Sample Diluent: 1 x 50 ml
7. Wash Buffer (20 x concentrate): 1 x 50 ml
8. TMB Substrate: 2 x 6 ml
9. Stop Solution: 1 x 7 ml
10. Adhesive Strip (For 96 wells): 3
11. Instruction manual: 1

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm.
2. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
3. Squirt bottle, manifold dispenser, or automated microplate washer.
4. Absorbent paper for blotting the microtiter plate.
5. 100ml and 500ml graduated cylinders.
6. Deionized or distilled water.
7. Pipettes and pipette tips.
8. Test tubes for dilution.

Storage

The unopened kit should be stored at 2 - 8°C. Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

Serum: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g, 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C.

Avoid repeated freeze-thaw cycles. Centrifuge the sample again after thawing before the assay.

Sample Dilution:

Dilute the serum or plasma samples with Sample Diluent (1:1000) before test. The suggested 1000-fold dilution can be achieved by adding 5µl sample to 95µl of Sample Diluent. Complete the 1000-fold dilution by adding 5µl of this solution to 245µl of Sample Diluent.

Note:

1. Creative Diagnostics is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 2 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

Note:

* Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.

* Bring all reagents to room temperature (18-25°C) before use for 30min.

* Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

HRP-conjugate (1x) - Centrifuge the vial before opening.

HRP-conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µl of HRP-conjugate + 990 µl of HRP-conjugate Diluent.

Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently

until the crystals have completely dissolved.

Dilute 25 ml of Wash Buffer Concentrate (20x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1x).

Assay Procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and controls be assayed in duplicate.

1. Prepare all reagents, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.
4. Add 100 µl of Negative Control, Positive Control or diluted Sample per well. Samples and controls must be assayed in duplicate. Cover with the adhesive strip provided. Incubate for 30 minutes at room temperature (20-25°C).
5. Aspirate each well and wash for a total of five washes. Wash by filling each well with Wash Buffer (350 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or Auto washer, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µl of HRP-conjugate (1x) to each well. Cover the microtiter plate with the adhesive strip. Incubate for 30 minutes at room temperature (20-25°C).
7. Repeat the aspiration/wash process for five times as in step 5.
8. Add 100 µl of TMB Substrate to each well. Incubate for 15 minutes at room temperature (20-25°C). Protect from light.
9. Add 50 µl of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
10. Take blank well as zero, determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

*Samples may require dilution. Please refer to Sample Preparation section.

Interpretation Of Results

For calculation the valence of human HPV16 L1 antibody (IgG), compare the sample well with control.

* While $OD_{\text{sample}} / OD_{\text{negative}} \geq 2.1$: Positive

* While $OD_{\text{sample}} / OD_{\text{negative}} < 2.1$: Negative

Precision

Intra-assay Precision (Precision within an assay): CV%<10%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<10%

Three samples of known concentration were tested in twenty assays to assess.

Specificity

This assay has high sensitivity and excellent specificity for detection of human HPV16 L1 antibody (IgG). No significant cross-reactivity or interference between human HPV16 L1 antibody (IgG) and analogues was observed. Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human HPV16 L1 antibody (IgG) and all the analogues, therefore, cross reaction may still exist.

Precautions

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Limitations

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
5. This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.