Creative Diagnostics®

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User's Manual

CVPx™ Mouse HPV 35 L1-VLP IgG ELISA Kit

REF **DEIA-JY2542**

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

Enzyme immunoassay for qualitative analysis of Mouse Anti-HPV-35 L1-VLP IgG in mouse serum samples.

General Description

HPV is a double-stranded DNA virus with a circular genome that encodes early genes, including E1, E2, E4, E5, E6, and E7, essential for replication, transcription, and transformation, and late genes L1 and L2, encoding viral capsid proteins. The replication cycle of HPV is tightly linked to the differentiation of the infected epithelium. Indeed, viral protein production and virus assembly occur only in the upper differentiated layers of the epithelium. In the basal layer, HPV particles initially interact with the basement membrane mostly through heparan sulfate proteoglycans (HSPGs)—capsid L1 contacts, and subsequently bind to HSPGs present on the basal keratinocyte cell surface. This attachment triggers conformational changes in the L2 capsid protein, resulting in exposure of a consensus cleavage site in the L2 N-terminus, whose proteolysis facilitates further interaction of the viral capsid with secondary receptor (s) present on the keratinocyte membrane. After such binding, HPVs are generally internalized by clathrin-dependent endocytosis, which initially relies on actin-rich cell protrusions, acting as the transport mechanism along the endocytic machinery.

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-mouse 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 mouse HPV35 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 Microplate: 1 (96 wells)
2. Negative Control (50×): 1 × 30 µl
3. Positive Control (50×): 1 × 30 µl
4. HRP-conjugate (100×): 1 × 120 µl
5. Sample & HRP-conjugate Diluent: 1 × 50 ml
6. Wash Buffer (20 ×): 1 × 50 ml
7. TMB Substrate: 2 × 6 ml
8. Stop Solution: 1 × 7 ml
9. Adhesive Plate Seals (For 96 wells): 3
10. Instruction manual: 1

Materials Required But Not Supplied

1. Microplate reader capable of reading absorbance at 450 nm.
2. Automated plate washer (optional).
3. Pipettes and pipette tips. Multichannel pipettes are recommended for a large numbers of samples.
4. Deionized or distilled water.
5. Test tubes for dilution.
6. ELISA plate shaker
7. 37°C incubator

Storage

The HRP-Conjugated Antibody Solution (100×), Negative Control (50×), and Positive Control (50×) should be stored at -20°C, while all other components of the kit should be stored at 2-8°C.

Specimen Collection And Preparation

Use mouse serum samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2-8 °C; otherwise, they should be aliquoted and stored deep-frozen (-80°C~ -20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

1. **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. Centrifuge the sample again after thawing before the assay.
2. **Sample Dilution:**

Dilute the Negative Control (50×)、Positive Control (50×)、mouse serum samples with Sample Diluent (1:50) before test.

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 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C or -80°C 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 (20-25°C) before use for 30 min.

* 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 by Sample Diluent. A suggested 100-fold dilution is 10 µl of HRP-conjugate + 990 µl of Sample Diluent.

Wash Buffer (1x) - If crystals have formed in the concentrated solution, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 ml of Wash Buffer Concentrate (20x) with deionized or distilled water to a final volume of 500 ml of Wash Buffer (1x).

Assay Procedure

Bring all reagents and samples to room temperature (20-25°C) 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 beassayed 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 (300 µl) sing a squirt bottle, multi-channel pipette, manifold dispenser, or Auto washer, and let it stand for 30 seconds, 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 (not to Blank!). 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 20 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

Cut-off = 0.1 + OD (Negative Control)

While ODsample ≥ cut-off: Positive

While ODsample < cut-off: Negative

Detection Range

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.

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.