Version 20-08/24

User's Manual

CVPxTM Human HPV 58 Antigen ELISA Quantitation Kit

REF **DEIASL121**

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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 immunoassay for quantitative analysis of HPV-58 antigen in 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 kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates. And the HRP conjugated antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450 nm in a microplate reader, and then the concentration of target can be calculated.

Reagents And Materials Provided

1. Coated Microplate: 1 (96 wells)
2. HPV 58 Antigen Standard: Lyophilized, 10 μg/mL, 1 vial.
3. HRP-Conjugated Ab Solution: 100×, 120 μL, 1 vial.
4. HRP-Conjugated Dilution Buffer: 12 mL, l vial.
5. Sample Diluent: Ready to use, 50 mL, 1 vial.
6. Washing Buffer: 20×, 50 mL, 1 vial.
7. TMB Substrate Solution: 6 mL, 2 vial.
8. Stop Solution: 7 mL, 1 vial.

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

All other components of the kit store at 2-8°C;

HRP-Conjugated Ab Solution 100× store at -20°C

Specimen Collection And Preparation

Use human serum or plasma (citrate, heparin) 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 (-70…-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.

Reagent Preparation

1. **1× Washing Buffer**: Dilute the 20× Washing Buffer to 1× Washing Buffer, 50 ml 20× Washing Buffer + 950 ml ddH_2O.
2. **HRP-Conjugated Antibody Working Solution**: Dilute the HRP-Conjugated Antibody Solution 100× to prepared HRP-Conjugated Dilution Buffer. Dilution ratio: 1:100, mix well.
3. **Standard:** Use 200 µL ddH_2O solution standard. It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Dilute the Standard with the prepared Sample Diluent. For example:

Callibrator	Callibrator Volume	Dilution Factor	Volume of Cal0
Stock-10 µg/mL*	200 µL ddH_2O		
Cal8 500 ng/mL	50 µL of stock*	1:20	950 µL
Cal7 250 ng/mL	0.5 mL of Cal8	1:2	0.5 mL
Cal6 125 ng/mL	0.5 mL of Cal7	1:2	0.5 mL
Cal5 62.5 ng/mL	0.5 mL of Cal6	1:2	0.5 mL
Cal4 31.25 ng/mL	0.5 mL of Cal5	1:2	0.5 mL

Cal3 15.625 ng/mL	0.5 mL of Cal4	1:2	0.5 mL
Cal2 7.8125 ng/mL	0.5 mL of Cal3	1:2	0.5 mL
Cal1 0 ng/mL	Sample Diluent	-	-

• The remaining standard should be stored at -20°C or below.

Assay Procedure

Preparations Before Assay. Please read the following instructions before starting the experiment.

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.

Assay Steps

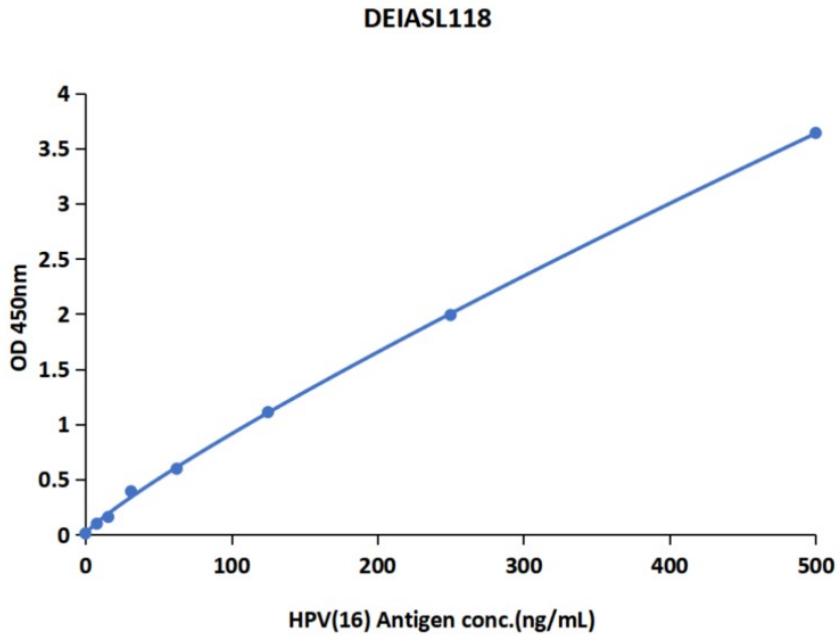
It is recommended that all reagents and materials be equilibrated to room temperature (20-25°C) prior to the experiment.

1. Prepare all reagents as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them vacuum-packed.
3. Add 100 µl of the standard, samples or control per well. Add 100 µl of the Sample Diluent into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 60 minutes at 37 °C incubator, 200 rpm.
5. Wash the plate 5 times with the 1× Washing Buffer.
6. Add 100 µL of the prepared HRP-Conjugated Antibody Working Solution to each well.
7. Cover with the plate sealer provided and incubate for 60 minutes at 37 °C incubator, 200 rpm.
8. Repeat step 5.
9. Add 100 µl of TMB Substrate Solution to each well. Cover with the plate sealer provided and incubate in the dark for 5-10 minutes at RT.
11. Add 50 µl of Stop Solution to each well. The color should immediately change to yellow.
12. Within 20 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450/630 nm.

Interpretation Of Results

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) versus concentration (log x-axis) for standards and fit the data with a four-parameter logistic equation. Using the equation of the line, calculate the concentration of analyte in each sample, making sure to correct for any sample dilution.



Precautions

1. For professional use only.
2. In case of severe damage to the kit package, please contact CD or your supplier in writing within one week of receiving the kit. Do not use damaged components in test runs, but keep them safe for complaint-related issues.
3. Obey the lot number and expiry date. Do not mix reagents from different lots, and do not use expired reagents.
4. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Ensure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.), please refer to the local distributor.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves, and protective glasses where necessary.
6. Reagents in this kit containing hazardous materials may cause eye and skin irritations.
7. Chemicals and prepared or used reagents must be treated as hazardous waste according to national biohazard safety guidelines or regulations.