Version 22-04/24

User's Manual

HPV (16) Antigen ELISA Quantitation Kit

REF **DEIASL118**

Σ **96T**

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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-16 antigen in samples.

General Description

HPV is a double-stranded DNA virus with a circular genome that encodes early-, including E1, E2, E4, E5, E6, and E7 essential for replication, transcription and transformation, and late-genes L1 and L2, encoding for viral capsid proteins. The replication cycle of HPV is tightly linked to differentiation of the infected epithelium. Indeed viral protein production and virus assembly occurs only in the upper differentiated layers of the epithelia. In wound 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 basal keratinocytes cell surface. This attachment triggers conformational changes in the L2 capsid protein, resulting in exposure of a consensus cleavage site in L2 N-terminus, whose proteolysis facilitates further interaction of viral capsid with secondary receptor(s) present on keratinocytes membrane. After such binding, HPVs are generally internalized by clathrin-dependent endocytosis, which is initially reliant 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. HRP-Conjugated Antibody Solution: 100×, 120 µl, 1 vial.
2. BSA: 3 g, 1.
3. Casein: 0.5 g, 1.
4. Washing Buffer: 20×, 50 ml, 1 vial.
5. Substrate Solution: 11 ml, 1 vial.
6. Stop Solution: 7 ml, 1 vial.
7. Positive Control: 0.1 mg/ml, 1 vial.
8. Plate Sealers: 2 Pieces.

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 capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
4. Multichannel pipettes are recommended for a large numbers of samples.
5. Deionized or distilled water.
6. 500 ml graduated cylinders.
7. Test tubes for dilution.
8. 37°C incubator.

Storage

2-8°C

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. **Dilute Buffer**: Dilute the BSA 3g to prepared 100 ml 1× Washing Buffer, mix well.
3. **HRP-Conjugated Dilution Buffer**: Dilute the Casein 0.5g to prepared 100 ml 1× Washing Buffer, mix well.
4. **HRP-Conjugated Antibody Working Solution**: Dilute the HRP-Conjugated Antibody Solution 100× to prepared HRP-Conjugated Dilution Buffer (3). Dilution ratio: 1:100, mix well.
5. **Standard**: It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Dilute the Standard 0.1 ng/ml with the prepared Dilute Buffer (2). For example:

   • Add 1244 µl Dilution Buffer and 6 µl Standard 0.1 ng/ml into one EP tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

   • Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the Sample Dilution Buffer into each tube. Add 0.3 ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.

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 (18-25°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 in the original packaging.
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. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Wash the plate 3 times with the 1× Washing Buffer.
7. Add 100 µL of the prepared HRP-Conjugated Antibody Working Solution to each well.
8. Cover with the plate sealer provided and incubate for 60 minutes at 37 °C incubator, 200 rpm.
9. Repeat step 5 and step 6.
10. Add 100 µl of 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 (Zero tube - 1/64 tube) 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.

Standards Concentration	OD		OD average
480ng/mL	3.205	3.356	3.281
240ng/mL	2.236	2.018	2.127
120ng/mL	1.377	1.345	1.361
60ng/mL	0.846	0.830	0.838
30ng/mL	0.481	0.436	0.459
15ng/mL	0.225	0.207	0.216
7.5ng/mL	0.124	0.107	0.116
NC	0.010	0.010	0.010

Precision

CVs ≤ 15% (n=10)

Detection Range

7.5 ~ 480 ng/ml

Detection Limit

≤ 7.5 ng/ml

Precautions

1. For professional use only.
2. In case of severe damage of the kit package please contact CD or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs but keep safe for complaint related issues.
3. Obey lot number and expiry date. Do not mix reagents of different lots. 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. Be sure 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. All reagents of this kit containing human serum or plasma (standards etc.) have been tested and were found negative for HIV I/II, HBsAg and Anti-HCV. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.
7. Reagents of this kit containing hazardous material may cause eye and skin irritations.
8. Chemicals and prepared or used reagents must be treated as hazardous waste according the national biohazard safety guidelines or regulations.