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User's Manual

HPV(52) Antigen ELISA Quantitation Kit

REF **DEIASL120**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

HPV(52) Antigen ELISA Quantitation Kit is designed for quantitatively detection of the contents of HPV-52 antigen in the samples.

General Description

Human papillomavirus (HPV) is a DNA virus from the papillomavirus family that is capable of infecting humans. Like all papillomaviruses, HPVs establish productive infections only in keratinocytes of the skin or mucous membranes. L1 is a major capsid protein of human papilloma virus. Infection with specific types of HPV has been associated with an increased risk of developing cervical neoplasia.

Principles of Testing

This product adopts the principle of Double Antibody Sandwich Method (Sandwich Elisa). The flat-bottom 96-well plates are coated with anti-HPV-52 antibody. After adding the samples, wash and remove the unconjugates. After adding the samples, wash and remove the unconjugates. Add another biotin-labeled anti-HPV52 antibody, incubate and wash. Then add HRP-labeled streptavidin to form an antibody-antigen-biotin-conjugated antibody-HRP-SA complex. Finally, the content of the HPV-52 in the samples can be detected by the degree of TMB color development.

Reagents And Materials Provided

1. Coated plate, 12 wells × 8 strips
2. Dilution buffer (for sample, biotin conjugate, and HRP-labeled streptavidin): 3% BSA +0.1Tween20
3. Biotin conjugate, 120μL×1 tube (diluted 100 times for use)
4. HRP-labeled streptavidin, 120μL×1 tube (diluted 100 times for use)
5. BSA, 3g × 1 pack
6. Tween20,100μl × 1 vial
7. 20× Washing Buffer, 50mL×1 vial
8. Substrate Solution, 11mL×1 vial
9. Termination Solution, 7mL×1 vial
10. Sealing plate film, 2 pieces
11. Instruction manual

Storage

1. All components remain stable under the condition of 2-8°C;
2. Avoid light. Valid for six months

Reagent Preparation

1. 1×washing buffer: Take 1 vial of 20×washing buffer, dilute it to 1000ml with deionized water, mix well for later use.
2. 0.1‰ washing buffer: Add 50μl Tween20 into 450ml 1×washing buffer (prepared in step 1), mix well.
3. Dilution buffer: Dissolve BSA (3g/pack) completely into 100ml of the prepared 1×washing buffer (step 1), mix well. Then discard 100μl mixture and add into 100μl Tween20, mix well.
4. Biotin conjugate solution: Take the required Biotin conjugate, dilute it 100 times with the dilution buffer prepared in step 3, mix well.
5. HRP-labeled streptavidin solution: Take the required HRP-labeled streptavidin, dilute it 100 times with the dilution buffer prepared in step 3, mix well.

Assay Procedure

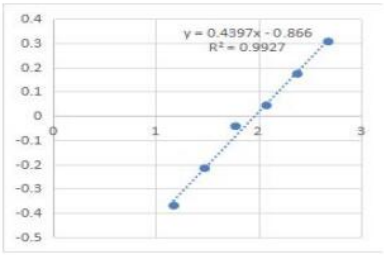
1. Equilibration: Equilibrate the required reagents at room temperature (18~25°C) for 30 minutes.
2. Adding standard and samples: Remove the coated plate from the sealed bag and dilute the standard to the different concentrations (using the dilution buffer prepared in "Reagent Preparation"). After adding 100μl of standard or sample to each well (including negative control), seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes.
3. Washing: Discard the liquid in each well, fill the microwells (350μl/well) with 1× washing buffer, and discard the liquid in the wells after 30 seconds. Repeat these steps for 3 times, then pat the plate on the paper towel after the last wash.
4. Adding biotin conjugate solution: Add the solution to the microplate (100μl per well). Seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes.
5. Washing: Discard the liquid in each well, fill the microwells (350μl/well) with 1× washing buffer, and discard the liquid in the wells after 30 seconds. Repeat these steps for 3 times, then pat the plate on the paper towel after the last wash.
6. Adding HRP-labeled streptavidin solution: Add the solution to the microplate (100μl per well). Seal the plate with sealing film. Place the plate in a shaking incubator (37°C, 200 rpm) and incubate for 60 minutes.
7. Washing: Discard the liquid in each well, fill the microwells (350μl/well) with 1× washing buffer, and discard the liquid in the wells after 30 seconds. Repeat these steps for 3 times, then pat the plate on the paper towel after the last wash.
8. Coloring: Add 100μl of Substrate Coloring Solution into each well. Mix well with gentle tapping. Then incubate the plate at room temperature for 10 minutes in the dark.
9. Termination: Terminate the reaction by adding 50μl of 0.2M H_2SO_4 into each well and mix gently. Set the main wavelength of the microplate reader at 450nm and the reference wavelength at 630nm. Measure the absorbance (OD value) of each well.

Typical Standard Curve

It is recommended to adopt the fitting method of LogX-LogY for fitting and calculation. After linearly fitting the logarithm value of concentrations with the logarithm of the corresponding OD values, the curve equation is as

follows:

Standard Curve Concentration	OD Value	
480	1. 946	2. 099
240	1. 451	1. 527
120	1. 07	1. 138
60	0. 915	0. 896
30	0. 6	0. 616
15	0. 441	0. 412
7. 5	0. 234	0. 224
NC	0. 074	0. 044



Precision

CV% ≤15% (n=10)

Detection Range

7.5~480ng/ml

Sensitivity

Sensitivity: ≤7.5ng/ml

Precautions

1. Avoid cross contamination.
2. Follow reader measure as a standard.
3. All samples and buffers should be added or removed with pipette.
4. Do not mix reagents from different batches.

Limitations

1. This kit is only used to detect the content of HPV-52 in samples.
2. Results out of the measurement range of the kit are unreliable.
3. Severe hemolysis, chyle, and bilirubin samples may cause abnormal test results.
4. This kit is developed for in vitro research only.