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Human Papilloma Virus-18 (HPV-18) Quantitation Kit (ELISA)

【Generic Name】

Human Papilloma Virus-18 (HPV-18) Quantitation Kit (ELISA)

Cat#: 17-0102

【Intended Use】

To detect the contents of HPV-18 antigen in the samples.

【Principle】

This product adopts the principle of Double Antibody Sandwich Method (Sandwich Elisa). The flat-bottom 96-well plates are coated with anti-HPV-18 antibody. After adding the samples, wash and remove the unconjugates. Add another biotin-labeled anti-HPV18 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-18 in the samples can be detected by the degree of TMB color development.

【Materials and Reagents】

1. Coated plate, 12 wells × 8 strips
2. Dilution buffer (for sample, biotin conjugate, and HRP-labeled streptavidin): 0.5% casein + 0.1% Tween20
3. Biotin conjugate, 120 μL × 1 tube (diluted 100 times for use)
4. HRP-labeled streptavidin, 120 μL × 1 tube (diluted 100 times for use)
5. Casein, 0.5g × 1 pack
6. Tween20, 200μl × 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 book

【Storage】

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

【Protocol for Detection】

1. Equilibration

Equilibrate the required reagents at room temperature (18~25°C) for 30 minutes.

2. Dosing solution: Please configure the reagents before use.

2.1 1× washing buffer: Take 1 vial of 20× washing buffer, dilute it to 1000ml with deionized water, mix well for later use.

2.2 0.1% washing buffer: Add 50 μl Tween20 into 450ml 1× washing buffer (prepared in step 2.1), mix well.

2.3 Dilution buffer: Dissolve Casein (0.5g/pack) completely into 100ml of the prepared 1× washing buffer (step 2.1), mix well. Then discard 100 μl mixture and add into 100 μl Tween20, mix well.

2.4 Biotin conjugate solution: Take the required Biotin conjugate, dilute it 100 times with the dilution buffer prepared in step 2.3, mix well.

2.5 HRP-labeled streptavidin solution: Take the required HRP-labeled streptavidin, dilute it 100 times with the dilution buffer prepared in step 2.3, mix well.

3. 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 step 2.2). 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

4. 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.

5. 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.

6. Washing

Repeat step 4.

7. 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.

8. Washing

Repeat step 4.

9. 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.

10. Termination

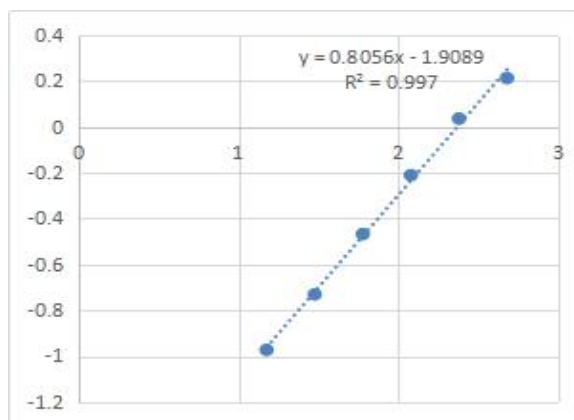
Terminate the reaction by adding 50μl of 0.2M H₂SO₄ into each well and mix gently. Set the main wavelength of the microplate reader at 450nm and the reference wavelength at 630nm. Measure the absorbance (OD value) of each well.

【Result estimate】

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	1.679
240	1.103	1.073
120	0.617	0.616
60	0.335	0.347
30	0.182	0.19
15	0.105	0.108
7.5	0.063	0.063
NC	0.027	0.026



【Product Performance Index】

1. Linear range: 7.5~480ng/ml
2. Sensitivity: ≤ 7.5 ng/ml
3. Accuracy: CV% $\leq 15\%$ (n=10)

【Limitations】

1. This kit is only used to detect the content of HPV-18 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

【Caution】

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.