



Pseudotyped RFP HPV 33 (PSVR-HPV33)

This product is for research use only and is not intended for diagnostic use.

PRODUCT INFORMATION

Product Overview	Virus-like Particle (VLP) Based on Human Papilloma Virus (HPV) Capsid Proteins L1 and L2
	This product is a virus-like particle (VLP) that self-assembles after the simultaneous expression of the capsid proteins L1 and L2 of different types of Human Papilloma Virus (HPV) in 293T cells. Additionally, it encloses a nucleic acid sequence containing a reporter gene within the pseudo-virus. The resulting pseudo-virus closely resembles the structure of a live virus and exhibits single-cycle infection cell activity, allowing for the expression of the reporter gene while maintaining good safety.
Specificity	HPV33
Species	Virus
Recommended Usage	Application Example (only for reference): Serum Neutralizing Antibody Detection Experiment
	Steps
	1. Cell Seeding
	• Take 293T cells, digest with trypsin, count, and prepare a cell suspension with a concentration of 1×10^5 cells/mL. Inoculate 100 μ L of cell suspension per well in a 96-well plate and continue cultivation in a 37°C, 5% CO ₂ cell culture incubator.
	2. Serum Preparation
	(a) Heat the serum samples in a 56°C water bath for 30 minutes (recommended for heat inactivation to remove complement interference).
	(b) Dilution: Dilute the serum samples with DMEM culture medium, perform a series of gradient dilutions for independent serum samples for neutralizing antibody detection.
	3. Pseudovirus Dilution
	• Retrieve pseudovirus from a -80°C ultra-low-temperature freezer and thaw on ice or at 4°C. After complete thawing, briefly centrifuge to collect the virus solution at the bottom of the

storage tube. Dilute the pseudovirus according to the pseudovirus titers from the report and experimental design (1 TU = 1 fluorescent spot). It is recommended to add 1000 TU pseudovirus solution per well in the 96-well plate.

- **Due to varying infection efficiencies of pseudovirus on 293T cells from different sources, a pilot experiment is suggested before the formal experiment to determine the optimal virus quantity.**

4. Pre-incubation of Test Serum Samples and Pseudovirus Diluent

- Mix the diluted test serum samples with the pseudovirus diluted in equal volumes. Incubate at 37°C, 5% CO₂ for 1 hour.

5. Cell Infection

- After incubation, slowly add 100 µL of the mixture to the corresponding wells of the 96-well plate with pre-seeded cells. Set up cell controls (DMEM culture medium) and pseudovirus controls (pseudovirus diluent mixed with DMEM culture medium in equal volumes). Gently scratch a "cross" pattern on the workbench to mix thoroughly. Incubate at 37°C, 5% CO₂ for 48h or 72 hours.

6. Infection Detection

- After 48h or 72h of virus infection, observe the expression efficiency of report protein.
- Detection Method 1 (for GFP/RFP/BFP): Use an Elispot fluorescent spot counter to calculate the titer.
- Detection Method 2 (for Luciferase): Using a professional luciferase assay kit for collection of samples and detection.

7. Result Interpretation

In the process of serum neutralizing antibody detection experiment, it is necessary to set pseudovirus controls and cell controls to determine the validity of the experiment.

- (For Luciferase) The inhibition rate is calculated as $1 - 100\% \times (\text{Mean value of sample group} - \text{Mean value of cell control group}) / (\text{Mean value of pseudovirus control group} - \text{Mean value of cell control group})$. The neutralizing antibody titer is represented by the reciprocal of the serum dilution corresponding to 50% inhibition rate.
- (For GFP/RFP/BFP) Or by performing titer analysis through cell counting of positive cells.

Note:

1. It is recommended to conduct a pilot experiment in advance to calculate the appropriate concentration of pseudovirus for use.
2. This product is intended for the in vitro detection of HPV antiserum or neutralizing antibody titers only.
3. Avoid repeated freezing and thawing of pseudovirus and serum samples.
4. When diluting the serum gradient, be cautious to avoid the formation of bubbles.
5. All experimental procedures must be conducted in a Biosafety Level 2 cabinet, and the waste generated during the experiment must be subjected to high-pressure sterilization treatment and subsequent processing according to medical waste disposal requirements.
6. The pseudovirus is of a small volume, so please briefly centrifuge it before use and then

proceed with sampling.

Size	100 μ L / 500 μ L
Storage	Store at -80°C. Multiple freeze/thaw cycles not recommended. When using the virus, transfer the virus from the -80°C refrigerator and melt it in an ice bath.
Ship	Frozen on dry ice

BACKGROUND

Introduction

HPV Pseudovirus and Neutralizing Antibodies

The series of HPV pseudoviruses contain viral antigens and have the ability to infect cells. Neutralizing antibodies can identify HPV pseudoviruses and initiate a neutralization reaction, reducing the infectivity of the HPV pseudovirus, leading to a decrease in the expression level of carried reporter genes such as RFP.

The Elispot reader can be used to scan and count the cell numbers of RFP positive spots in each well, or a Chemiluminescence detector to read the expression level of RFP in each well. By comparing the values of the test sample wells with the values of the pseudovirus control group, the inhibition percentage of the test sample can be calculated.