HPV (16) GFP Pseudovirus

Catalog # PPL16-PGE045

Dsecription

Human papillomavirus (HPV) is a common sexually transmitted infection (STI), which is related to several diseases, such as genital warts and tumours. The virus has no envelope and uses major capsid protein (L1) and Minor capsid protein (L2) as its capsid with a double-stranded circular DNA inside.

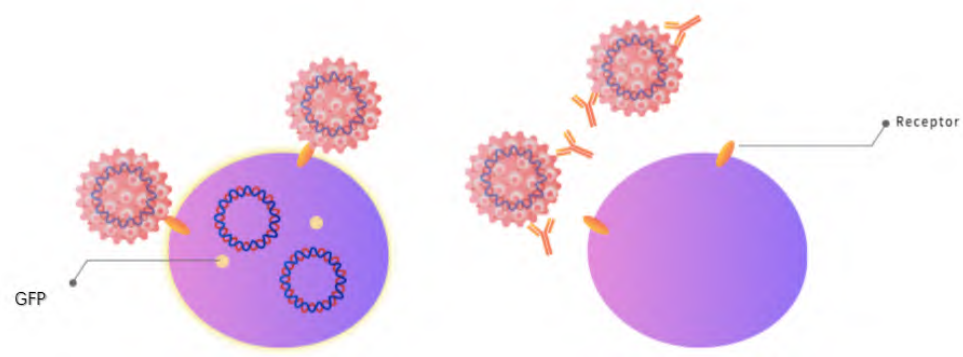
HPV (16) GFP Pseudovirus is packaged with L1 and L2 proteins of HPV type 16 expressed in SV40 Large T antigen overexpressing HEK 293T cells, as well as a double-stranded DNA containing GFP gene. The pseudovirus can mimic the structure of HPV, while does not contain oncogenes from HPV, such as HPV E6 / E7, and replication deficient. It can effectively infect SV40 Large T antigen overexpressing HEK 293T cells and can be used in determining neutralizing antibody titer, screening for inhibitors, studying virus invasion and HPV vaccine development.

The reference sequences are: Uniport # P03101 (L1) and Uniport # P03107 (L2).

Pseudovirus Profile

Product description	HPV (16) GFP Pseudovirus
Envelope	N.A.
Capsid protein	HPV type 16 L1 / L2
Reporter	GFP
Physical appearance	Green liquid
Storage	Below -70°C for up to 1 year from date of receipt
Transport	Dry ice
Application	Neutralization assay

Schematic Diagram (Neutralization)



Protocol (Pseudovirus Neutralization Assay)

a. Mix 89% DMEM medium, 10% Fetal bovine serum and 1% Penicillin-Streptomycin to prepare complete DMEM medium.

b. Thaw the pseudovirus at room temperature. Dilute the pseudovirus with complete DMEM medium **according to your pre-test results**. In general, we recommend a 100-fold to 1000-fold dilution, among which 1000-fold dilution is optimal at ACROBiosystem's experimental platform

c. Dilute your samples with complete DMEM medium in a 96-well plate (flat bottom clear, black polystyrene) to reach a volume of 75 μL per well, then add 25 μL pseudovirus dilution per well to reach a final volume of 100 μL per well. Gently flap to mix well. Incubate the plate at suitable temperature for 60 min. We suggest that 4°C is an optimal incubation temperature, while 37°C is an alternative .

d. Digest and resuspend SV40 Large T antigen overexpressing HEK 293T cells with complete DMEM medium. Adjust the cell density to 5×10^5 cells per milliliter with complete DMEM medium. Seed 100 μL the cell suspension per well into the 96-well plate. Gently flap to mix well. Incubate the plate in a 5% (vol/vol) CO_2, 37°C incubator for 48 h.

e. Take out the 96-well plate and read the GFP values of the wells with the related instrument.

f. Calculate the inhibition rate with the following formula:

$$\textbf{Inhibition rate} = \left(1 - \frac{X - \overline{CC}}{\overline{VC} - \overline{CC}}\right) \times 100\%$$

X: the GFP value of a certain well;

CC: cell control, only cells are added;

$\overline{CC}$: the mean value of cell control group;

VC: virus control, only cells and pseudovirus are added;

$\overline{VC}$: the mean value of virus control group.

Notice

a. Though pseudovirus particles has no pathogenicity and cannot replicate, the assays should be carried out carefully in a Biosafety Level 2 or higher-level laboratory with a biosafety cabinet.

b. Serum samples from animals or humans should be inactivated in a water bath at 56°C for 30 min before being tested.

c. The product is for **Research Use Only**.

Further Advice

How to get an ideal signal?

a. Please try to **avoid freezing and thawing**, which would influence the titer of the pseudovirus. **Please contact us if it is necessary for you to aliquot the product.**

b. HPV particles tend to adsorb on the surface of the container and aggregate. For this product, the buffer and low temperature can protect HPV particles against surface adsorption and aggregation. As a result, keep it below -70°C until use, dilute the pseudovirus suspension just before use and try to aviod change the container for storage.

c. There is a small quantity of surfactant in the pseudovirus suspension, which could influence the cell viability. As a result, we suggest that the addition volume should not be more than **1 μL pseudovirus suspension per well** of 96-well plate to avoid influencing the cell states.

How to keep safety?

a. Though HPV pseudovirus has no pathogenicity and oncogenes, there are still potential risks based on its ability to infect cells. Do not touch or intake the pseudovirus suspension, and all operations should be carried out cautiously.

b. Try your best to reduce the production of aerosol in the process of experiment.

c. Do not open pseudovirus-contaminated containers and use the pseudovirus out of well-run AII or BII biosafety cabinets.

d. HPV paticles are more stable than several frequently-used virus paticles, for example, lentivirus. Hence, strict disinfection procedures should be carried out.

f. Chlorine-containing disinfectant is efficient to inactivate HPV. Pseudovirus -contaminated wastes should be soaked in it at least overnight, and then be further processed with moist heat sterilization. In particular, HPV paticles can bear medicinal alcohol at a certain extent. As a result, the time of disinfection should be lengthened if alcohol is used for sterilization. What's more, alcohol should not be the only sterilization method.

g. The time of ultraviolet sterilization for the biosafety cabinets should be extended to at least an hour because of the tolerance of HPV paticles. Ultraviolet and ozone sterilizations should be used to keep the laboratory safe.