HPV (58) Fluc Pseudovirus

Catalog # PPL58-PLE044

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.

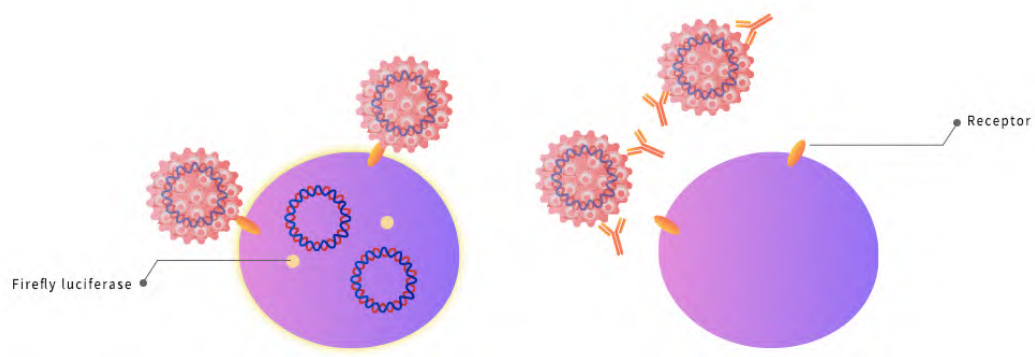
HPV (58) Fluc Pseudovirus is packaged with L1 and L2 proteins of HPV type 58 expressed in SV40 Large T antigen overexpressing HEK 293T cells, as well as a double-stranded DNA containing firefly luciferase gene. The pseudovirus can mimic the structure of HPV, while does not contain oncogenes from HPV, 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 # P26535 (L1) and Uniport # P26538 (L2).

Pseudovirus Profile

Product description	HPV (58) Fluc Pseudovirus
Envelope	N.A.
Capsid protein	HPV type 58 L1 / L2
Reporter	Firefly luciferase
Physical appearance	Colourless transparent to milk white 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 25-fold to 250-fold dilution, among which 250-fold dilution is optimal at ACROBiosystem's experimental platform.

c. Dilute your samples with complete DMEM medium in a 96-well white flat bottom plate 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. Prepare firefy luciferase detection reagent (britelite plus Reporter Gene Assay System (PerkinElmer, Cat. No. 6066761)) and balance it to room temperature.

f. Take out the 96-well plate and discard 100 μL medium per well, which makes there is approximately 100 μL medium left in each well. Balance the plate to room temperature for 10 min. Add 100 μL detection reagent and mix well. Incubate for 2~5 min at room temperature.

g. Read the luminescence values (RLU) of the wells with a luminescence meter (PerkinElmer, Cat. No. HH34000000).

h. 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 luminescence value (RLU) of a certain well;

CC, cell control, only cells are added;

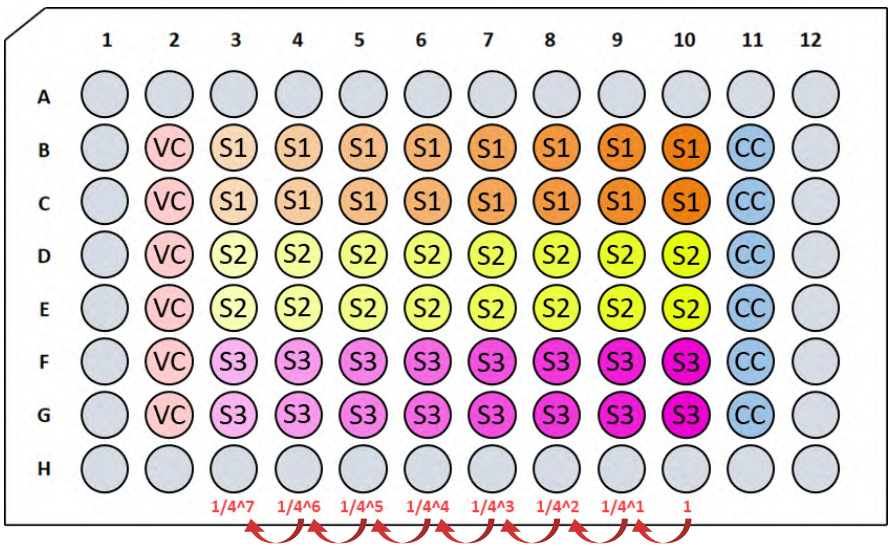
$\overline{CC}$, the mean value of cell control group;

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

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

Sample dilution with a 96-well plate

Mutiple layouts and dilution strategies are acceptable for pseudovirus neutralization assay. There is an example of "3 samples & 4-fold dilution":



a. Add 200 μL PBS per well into the gray wells to reduce evaporation.

b. Add 75 μL medium into well B2-G9, 100 μL medium into well B11-G11.

c. Add 100 μL samples diluted by medium into well B10-G10. The concentration of the sample should be 4/3 fold of the first expected gradient.

d. Pipet 25 μL with a multichannel pipettor from each of well B10-G10, into well B9-G9, do blowing-suction for 10 times.

e. Pipet 25 μL from well B9-G9, into well B8-G8, do blowing-suction for 10 times. The rest can be done in the same manner till well B3-G3.

f. Pipet and discard 25 μL from well B3-G3.

g. Add 25 μL pseudovirus dilution per well into well B2-G10, Gently flap to mix well, and go ahead with the following steps.

h. An effective antibody, positive serum and heparin sodium can be used as a positive control for HPV neutralization assay.

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.

d. The luminescence value (RLU) could be influenced by multi-factors. If **cells, reagents or luminescence meter** other than those in this protocol is used, a pre-test should be carried out to determine an appropriate pseudovirus dosage.

e. After addition of luciferase detection reagent and 2~5 min incubation, the signal values should be read **immediately** and the temperature should be around room temperature. At the same time, as FBS and phenol red show impact on the light output, discard all medium, then add 100 μL PBS and 100 μL detection reagent if you need a higher luminescence value (RLU).

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.

Notice:

a. All data above represent the general characters of this product. Please check the COA of a certain lot to find the exact titer and signal intensity.

b. For help, more information and protocols of related assays, please contact our technical support team at: ***TechSupport@acrobiosystems.com***.