Material and Method – Infection and Evaluation of Pseudovirus Function

1. Fluorescence observation by SRIP infection of Vero cell

Purpose

From the HEK 293T lipofected fluorescent gene + replicon in the previous experiment, Vero cells were infected with the virus like body produced in the culture supernatant: SRIP (single infectious virus), thereby expressing the fluorescent protein and observing it with a fluorescence microscope .

We also estimate the concentration of virus like body: SRIP.

Overview

Since the concentration of SRIP produced from HEK293T is unknown, try infecting vero cells and use the concentration at which 50 to 100 cells are infected per well of a 96-well plate and fluorescence is seen as a standard. If it is less than this, no significant difference can be obtained in the neutralization assay. If it is too much, the cell count becomes serious.

In the first experiment, concentrations are investigated by infecting the virus particle containing solution with three dilutions of × 2, × 20, × 100.

Preparation

- Vero cells
- 10% FBS DMEM, (Non-Essential AA), P / S
- Sample (SRIP & Serum)
- 96 well plate # 3596 Corning
- 96 well Round Bottom plate # 3799 Corning
- 96 well Half-Area plate white # 3696 Corning
- PBS
- Passive Lysisi Buffer # E 1941 Promega
- 96 plate shaker
- multi-pipet, multi-channel aspirator
- Fluorescence microscope

Day -1 (Day before infection)

① Put Vera cells of 1.2 × 10 4 [/ 100 μL] on a 96-well plate.(So as to be 90-100% confluent on the day of infection)

② Dilution of SRIP sample (× 2, × 20, × 100)

③ 96 well plate on ice, over night at 4 ° C

Day 0 - SRIPs infection

① Remove Vero cell supernatant

② Add 40 μL of SRIP per well. (Ribavirin as a control: add antiviral drug)

③ 37 ° C, 5 to 6 h incubation with CO 2 incubator

④ Remove inoculum (SRIP) (To suppress back, because you see with GFP, you do not have to do this time)

⑤ Add 100 μL / well of fresh medium

Day 2 or 3

Detection of fluorescence (infection)

The virus-like particle concentration that 50 to 100 cells can see cells infected in 1 well is set as a standard.

Observation with fluorescence microscope, cell count (keep model number etc. of microscope in mind)

2. Neutralization assay (probably not in time)

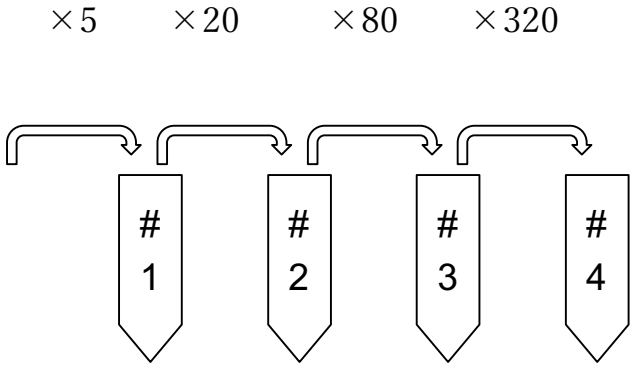
Purpose

SRIP prepared in the previous experiment is mixed with serum (including antibody against dengue virus) to remove SRIP reactive to the antibody. By infecting the Vero cells with this, it is confirmed that SRIP-derived fluorescent protein not reacting with the antibody is expressed and not from antibody-added fluorescent protein.

Procedure

Preparation of SRIPs / Serum mixture

① Dilute Serum (5 times at the beginning, then 4 times dilution)



② Dilution of SRIP sample (50 to 100 [Infectious unit / well]) → Next, put it in the same amount as Serum, so 50 to 100 [Infectious unit / 20 μl]

③ Mix SRIP and Serum in a 96-well plate (U-shape bottom)

Day 0 SRIPs infection

① Remove Vero cell supernatant

② Add 40 μL of Mixture (SRIP + Serum) per well.

③ 37 ° C, 5 to 6 h incubation with CO 2 incubator

④ Remove seed bacteria (SRIP + Serum) (To suppress backing, you do not have to do this time as you see it with GFP)

⑤ Add 100 μL / well of fresh medium

Day 2 or 3

Detection of fluorescence (infection)

Observation with fluorescence microscope, cell count (keep model number etc. of microscope in mind)

Reference - Luciferin Assay nano-Glo Luciferase assay # N1120 Promega

① Medium removal

② Add 300 μL / well PBS (wash)

③ Remove PBS

④ Add 30 μL / well of 1 × lysis buffer

⑤ After leaving for 15 minutes at room temprature, 20-30s Vortex

⑥ Insert Substrate at 10 ul / well in Half-Area plate white (# 3696 Corning)

⑦ Add 10 μL of lysing solution, add 20-30 s Vortex

⑧ Fluorescence measurement (Glomax microplate reader)