Expansion of DENV stocks

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Working
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MATERIALS

NAME	CATALOG #	VENDOR
TrypLE™ cell dissociation reagent	12605010	Life Technologies

STEPS MATERIALS

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Expansion of Cell Culture

1 Warm culture media and TrypLE at 37oC

TrypLE™ cell dissociation reagent
by Life Technologies
Catalog #: 12605010

2 From a 25cm2 bottle, remove media, wash with 2mL PBS and add 2mL TrypLE

3 Incubate for 10-15 min
00:10:00

4 After 10 minutes incubation, inspect for cell dettachment at microscope
Note: Some cell types might require pipetting of the cell media for complete dettachment.

5 Transfer dettached cells to a 225cm2 bottle containing 55 mL of cell media. Triturate against bottom of flask 8 - 10 times to break up cell clumps.

6 Incubate cell culture until they reach a confluence of 80-90%.

Virus infection of Vero Cells for Expansion

7 Check cell monolayer confluency. 80 – 90% confluency is optimal. Warm culture medium to 37°C.

8 Rapidly thaw virus stock vial in 37°C water bath. Vortex well to mix.
Note: Transfer the virus on ice between the rooms

9 Dilute virus in 5 mL of fresh culture medium to yield MOI = 0.01 (approximately 4 x 10^5pfu of virus, assuming 3 – 4 x10^7cells/flask). MOI can be 0.1 – 0.01 with little difference in final virus titer.

10 Remove medium from flask(s) to be infected.

11 Add 5 mL of virus dilution directly to cell monolayer. Rock flask back and forth to distribute inoculum.

12 Incubate flask at 32°C (or room temperature) for 5 – 10 min to maximize virus absorption. Rock flask back and forth occasionally. No need to remove inoculum.
00:10:00

13 Add 55 mL fresh culture medium to each flask.

14 Incubate 35 - 37°C, 5% CO_2, 80% relative humidity for 5 – 6 days. Gently rock flask(s) each day if possible to maximize virus spread and final virus titer.

Virus harvest and processing from Vero cell expansion

15

Note: By day 5, some CPE may be visible, but such CPE is strain specific. In most cases, CPE is not visible, yet virus titers will be high. In fact, virus titer usually peaks before CPE becomes apparent. Because CPE is minimal, harvested virus fluid will contain few cells and does not require clarification.

16 To each flask to be harvested, add 6 mL 10X SPG. Triturate with pipette to mix.

17 Filter virus fluid through 0.2 mm PES (low protein binding) filter unit. This removes cells, breaks up virus clumps, and ensures bacterial sterility.

18 Dispense virus fluid into labelled cryovials, 1.0 – 1.5 mL per tube.

19 Flash freeze vials in dry ice for about 10 min.

20 Place vials in freezer inventory box and store at -70°C or below.

After harvesting fluid, flask(s) can be re-fed with 60 mL fresh culture medium, returned to incubator, and re-harvested after two days. Repeat again if cell monolayer is intact.

Virus titration is usually performed before utilization of the viruses

21