Yellow Fever Virus replication in cell culture

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Abstract

In order to increase the number of viral particles a blind passage of sera might be performed in *Aedes albopictus C6/36* cells.

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Materials

Fetal Bovine Serum, qualified 10437-028 by Life Technologies

✓ PBS by Contributed by users

Leibovitzs L-15 Medium L4386 by Sigma Aldrich

Falcon® Serological Pipettes, 2 mL 1000 Pipettes 38002 by Stemcell Technologies

Cell culture tubers 91106 by Techno Plastic Products (tpp)

C6/36 cells by ATCC

28°C incubator without CO2 by Thermo Fisher Scientific

✓ Invert Microscope by Contributed by users

Falcon® Serological Pipettes, 5 mL by Stem Cell Technologies

Protocol

Step 1.
Remove and discard the cell culture media from the culture vessel.

Step 2.
Wash cells using PBS (approximately 2 mL per 10 cm2 culture surface area). Gently add wash solution to the side of the vessel opposite the attached cell layer and wash the cells.

Step 3.
Shaking the flask slightly and using a pipette resuspend the cells in a minimal volume of pre-warmed Leibovit'z with 5% BFS and remove a sample for counting.

Step 4.
Using a cell counter, determine the number of cells/mL and adjust the number of cells to 65,000 C6/36 cells/mL.

Step 5.
Inoculate in the bottom of a cell culture tube 100,000 C6/36 cells in 1.5 mL of Leibovit'z medium supplemented with 5% BFS.

Step 6.
Keep the tubes inclined to 45 degrees at 28oC.

Step 7.
After 12-16 hours, remove and discard the media using a sterile pipette.

Step 8.
Wash the cells twice with phosphate buffered saline.

Step 9.
Add 100 µl of a solution containing 20 µL of sera and 80 µL of Leibovit'z medium.

Step 10.
Incubate the cells for 1 hour at 28oC.

Step 11.
After adsorption, add 1,5mL of Leibovit'z medium supplemented with 2% BFS and incubate the cells at at 28oC.

Step 12.
Observe the cells daily in an inverted microscope up to the observation of cytopathic effect or up to 10 days.

Step 13.
Collect the supernatant, make aliquots and kept them at -70°C. You can use this supernatant for other blind passages aiming viral isolation or to perform RNA extraction and further molecular analysis.