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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.

MATERIALS

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⊗ Fetal Bovine Serum, qualified **Life Technologies Catalog #10437-028**

⊗ PBS **Contributed by users**

⊗ Leibovitzs L-15 Medium **Sigma Aldrich Catalog #L4386**

⊗ Falcon® Serological Pipettes, 2 mL 1000 Pipettes **Stemcell Technologies Catalog #38002**

⊗ Cell culture tubers **Techno Plastic Products (tpp) Catalog #91106**

⊗ C6/36 cells **ATCC**

⊗ 28°C incubator without CO2 **Thermo Fisher Scientific**

⊗ Invert Microscope **Contributed by users**

⊗ Falcon® Serological Pipettes, 5 mL **Stem Cell Technologies**

Protocol status: Working
We use this protocol and it's working

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- 1 Remove and discard the cell culture media from the culture vessel.
- 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.
- 3 Shaking the flask slightly and using a pipette resuspend the cells in a minimal volume of pre-warmed Leibovitz with 5% BFS and remove a sample for counting.
- 4 Using a cell counter, determine the number of cells/mL and adjust the number of cells to 65,000 C6/36 cells/mL.
- 5 Inoculate in the bottom of a cell culture tube 100,000 C6/36 cells in 1.5 mL of Leibovitz medium supplemented with 5% BFS.
- 6 Keep the tubes inclined to 45 degrees at 28°C.
- 7 After 12-16 hours, remove and discard the media using a sterile pipette.

- 8** Wash the cells twice with phosphate buffered saline.
- 9** Add 100 µl of a solution containing 20 µL of sera and 80 µL of Leibovitz medium.
- 10** Incubate the cells for 1 hour at 28°C.
- 11** After adsorption, add 1,5mL of Leibovitz medium supplemented with 2% BFS and incubate the cells at 28°C.
- 12** Observe the cells daily in an inverted microscope up to the observation of cytopathic effect or up to 10 days.
- 13** Collect the supernatant, make aliquots and keep 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.