MDCK culturing and splitting

HOST-PATHOGEN INTERACTIONS LAB
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Background

MDCK II (Madin-Darby Canine Kidney [Cocker Spaniel]) are polarized epithelial cells. They proliferate in culture until a perfect monolayer is created and then they stop to proliferate due to contact inhibition.

Culturing Reagents

Growth Medium formulation:

MEM-E (Minimum Essential Medium-Eagle, BI 01-025-1A) supplemented with 5% FBS (BI 04-007-1A), 1% L-Gln (200 mM, Stable Dipeptide, BI 03-022-1B) and 1% PSA antibiotics (BI 03-033-1B).

Splitting Reagents

Trypsin-EDTA Solution A (BI 03-050-1A)

DPBS (BI 02-023-1A)

Subculture Routine (recommended by ATCC)

Split sub-confluent cultures (70-80%) 1:5 twice a week i.e., seeding at 5 x 10,000 cells/cm^2 using 0.25% tryspin or trypsin/EDTA; 5% CO_2 37°C,

Start with 10 cm plate with 70-80% confluent MDCK monolayer.

1. Wash cells with 10-14 ml DPBS. Aspirate the buffer.
2. Add 1.5-2.0 ml of Trypsin A, agitate plate so the trypsin will cover the entire plate, and incubate at the CO_2 incubator for up to 10 min. Flip the plate gentry to detach the cells. Take a look at the microscope. If most of cells are detached, proceed to the next step. If not, place the cells back in the incubator for additional 2-3 min. It is very important not to over-trypsinize.
3. Add 8 ml of growth medium and resuspend the cells by passing them at least 5 times through a 10 ml pipette.
4. Place the cell suspension in a 10 ml conic tube, and spin 2000 rpm for 2-3 min.
5. Aspirate medium but leave some above the cell pellet.
6. Re-suspend the cells in that medium by continuous harsh flicking the edge of the tube.
7. Add 10 ml of growth medium and further resuspend the cells by passing through a 10 ml pipette at least 5 times.
8. For 1:10 splitting, take 1 ml of the suspended cells and place on the center of fresh 10 cm dish. Then add 9 ml of fresh medium to these cells and agitate the plate gently until all cells are spread evenly. Place in the CO_2 incubator.

Important notes:

Write your name and date of splitting on the dish's lead.

MDCK cells should be fed every 3 days and the cells should not be kept in culture at 100% confluence.

You should thaw fresh cells every 4-6 weeks or after 9 passages.