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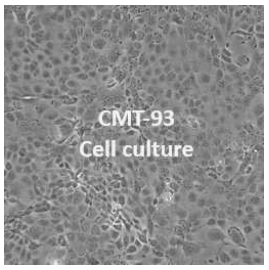
CMT-93 Cell Culture Protocol



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We are still developing and optimizing this protocol

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Abstract

CMT-93 is a cell line exhibiting epithelial morphology that was isolated from the rectum of a mouse with polyploid carcinoma.

Image Attribution

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Guidelines

Working with cell cultures requires a laminar flow cabinet. It has to be radiated with UV light, cleaned with any highly effective terminal disinfectant (such as Tego® 2000 or Suredis®) and 70% ethanol. All material introduced into the cabinet must also be sprayed with ethanol.
Once the work is finished, we must clean the cabinet with the detergent, then with 70% etOH and turn on the UV light for 30 min.

Materials

Plasticware:
p60 cell culture plates
p100 cell culture plates
Cell culture flasks, 75 cm^2, treated for cell attachment.
15 and 50 mL centrifuge tubes
Cryovials

To prepare the complete medium:
DMEM 1X
Fetal bovine serum heat inactivated (FBS)
Glutamine 200 mM

To subculture the cells:
Trypsin-EDTA
PBS 1X

Troubleshooting

Safety warnings

Every reagent must be sterile in order to avoid contaminations.

Before start

Clean and prepare the laminar flow cabinet, turn on the water bath and warm up the culture media.

Preparation of complete growth medium (DMEM+)

1 Add 445 mL 1X DMEM, 50 mL FBS and 5 mL glutamine [M] 200 millimolar (mM) to a sterile 500 mL bottle and homogenize

2 Label the bottle with name, group, phone number, date and additions.

3 Close with parafilm and store at 4 °C .

Cell thawing procedure

4 Remove one vial of cell stock from the liquid nitrogen tank with gloves and forceps. Transfer them to the cell culture laboratory in an appropiate container or a box with ice.

5 Thaw the vial by gently shaking it in a 37 °C water bath. Thawing should be rapid (approximately 2 min).

6 Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol

7 Transfer the contents of the vial to a centrifuge tube containing 9 mL of complete culture medium and 1200 rpm, Room temperature, 00:05:00 5m

8 Resuspend with 10 mL DMEM+ and distribute on 2 P60 plates.

9 Incubate cultures at 37 °C , 5% CO_2 Overnight 5m

Subculturing procedure

10 Remove and discard culture medium.

11 Rinse with PBS 1X solution and discard

12 Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. Discard.

13 Add 2 mL of Trypsin-EDTA solution to flask and incubate 00:10:00 at 37 °C to facilitate detachment from the plate. 10m

Note

To avoid clumping, **do not agitate** the cells by hitting or shaking the flask while waiting for the cells to detach.

14 Observe cells under an inverted microscope until cell layer is dispersed.

Note

If the cells are not detached already, incubate 00:05:00 more at 37 °C.

15 Add 5 mL of DMEM+ and aspirate cells by **gently** pipetting. Pour the existing volume down the walls of the flask in order to drag and collect as many cells as possible.

16 Collect the cell suspension in a centrifugue tube and 1200 rpm, Room temperature, 00:05:00. 5m

17 Discard the supernatant into a beaker with 70% EtOH or 10% bleach.

18 Resuspend in medium according to the dilution to be made.

Note

A subcultivation ratio of 1:4 to 1:10 is recommended

19 Add 1 mL of the cell supension to new culture vessels containing 14 mL DMEM+.

20 Incubate cultures at 37 °C, 5% CO_2 Overnight 5m

Cryopreservation and storage procedure 1d

21 Repeat steps of *Subculturing procedure* until the "Resuspend in medium according to the dilution to be made" step.

22 Resuspend in medium taking into account that for every p100 we can storage up to 2 cryovials of cells, containing 1 mL.

23 Prepare the cryovials with 50 µL DMSO.

24 Add 950 µL of the cell suspension to every cryovial.

25 Label the cryovials with cell line, passage, date and lab number or phone number.

26 Store the cryovials in a slow freezing container at -80 °C for 24:00:00. 1d

27 Transfer the cryovials to the liquid nitrogen tank.

Protocol references

https://www.atcc.org/products/ccl-223#detailed-product-information