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Cell subculture

PMAT0001 [1]

[1]Nanyang Technological University

1 *Works for me* This protocol is published without a DOI.

LKC Translational Neuro

PMAT0001

PROTOCOL CITATION

PMAT0001 2020. Cell subculture. **protocols.io** https://protocols.io/view/cell-subculture-bnk9mcz6

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GUIDELINES

- For every 25cm2 of surface area, fill with **5 mL** media
- Use about **10 mL** to **15 mL** of media for 75cm2
- PBS used is 1X
- 10% FBS in DMEM (**50 mL**)
- 1% PenStrep in DMEM (**5 mL**)

MATERIALS

NAME	CATALOG #	VENDOR
Trypsin	TB0626.SIZE.1g	Bio Basic Inc.
Fetal bovine serum		
Dulbecco's Phosphate Buffered Saline	D5652	Sigma Aldrich
Penicillin Streptomycin	15140 122	Invitrogen - Thermo Fisher
DMEM	11885	Invitrogen - Thermo Fisher

BEFORE STARTING

- Thoroughly wipe down hood and any item introduced into the hood with 70% ethanol
- Carry out asceptic techniques while working

15m

1 Pre-warm reagents to **37 °C** in water bath for about **00:15:00** .

2 Aspirate spent culture media from cell culture vessel.

3 Wash cells once with PBS (**2 mL** is enough to wash T25 flasks and maybe **5 mL** for T75 flasks).

4 Aspirate PBS (from the side of the plate that does not have any cells, so as to avoid disturbing the cells).

5 Add **2 mL** of Trypsin-EDTA (Add this volume for T25 flasks and accordingly about 5mL for T75 flasks) in the cell culture flasks; Incubate flasks for about **00:01:00** . 1m

6 After incubation, examine cells under a microscope. Fully trypsinized cells should appear rounded up and no longer attached to the surface of the flask. A few taps can be applied onto the flask to make cells detach faster.

7 Once the cells have detached, add FBS/serum-containing medium to the flask in an equal ratio to the added trypsin. Tyrypsin will start acting on excess serum proteins instead of harming cells.

8 Collect harvested cells and pipette into an appropriate-sized centrifuge tube.

9 Centrifuge cells for **00:05:00** at 1500rpm. 5m

10 After centrifuging, aspirate media and trypsin.

11 Add **2 mL** of fresh media into the tubes.

12 In each of the new flask (T25), add **5 mL** of fresh media.

13 Add about **1 mL** of cells from step 11 into each of the new flasks.

Rule of Thumb for ratio of splitting

14 - After the cells are 80% confluent, we should split them as they are in the log phase of growth
- Refer to attached images for the rule of splitting


Screenshot 2020-10-20 at 13.34.28.png


Screenshot 2020-10-20 at 13.35.08.png