Version 2

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Cell seeding V.2

PMAT0001 [1]

[1]Nanyang Technological University

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

PMAT0001

PROTOCOL CITATION

PMAT0001 2020. Cell seeding. **protocols.io** https://protocols.io/view/cell-seeding-bnp8mdrw Version created by PMAT0001

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MATERIALS

NAME	CATALOG #	VENDOR
1X Dulbecco's Phosphate Buffered Saline (DPBS)	14190094	Thermo Fisher Scientific
Penicillin Streptomycin	15140 122	Invitrogen - Thermo Fisher
DMEM	11885	Invitrogen - Thermo Fisher
Foetal Bovine Serum (FBS) Triple 0.1 µm Sterile Filtered 500 ml Australian Origin	FBS-AU-015	Serana

BEFORE STARTING

- Asceptic techniques***
- Wipe down hood and every item introduced into the hood with 70% ethanol

1 Take cells from the freezer and thaw for **00:02:00** in the **37 °C** water bath. 2m

2 Take a T75 plate and place all needed items in ethanol-sterilized laminar flow hood.

3 Draw the needed amount of cells into a 50mL centrifuge tube.

4 Add about **7 mL** of PBS into the centrifuge tube.

3m

5 Spin the mixture in a centrifuge for **00:03:00** at 1500rpm.

6 Decant supernatant and add PBS to the cell pellet once again.

7 Resuspend cell pellet with the PBS using a micropipette.

8 Repeat steps 5 to 7.

9 Add DMEM (with FBS and antibiotics) into centrifuge tube and resuspend cell pellet.

10 Add DMEM to the T75 plate (sufficient to cover the entire surface area, about **5 mL**).

11 Add **5 mL** of the cell mixture into the T75 flask.

12 Incuabte T75 flask at **37 °C** , 5% CO_2.