Version 2

Oct 21, 2020

Cell cryopreservation 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 cryopreservation. **protocols.io**
https://protocols.io/view/cell-cryopreservation-bnp7mdrn
Version created by PMAT0001

CREATED

Oct 21, 2020

LAST MODIFIED

Oct 21, 2020

PROTOCOL INTEGER ID

43487

GUIDELINES

- This protocol is for ADHERENT CELLS

MATERIALS

NAME	CATALOG #	VENDOR
Cell counter		Thermo Fisher Scientific
DMSO	9224	J.T. Baker
Bright-Line Hemacytometer	02-671-5	Fisher Scientific
Trypan Blue Stain 0.4%		Invitrogen - Thermo Fisher

MATERIALS TEXT

- Use a DMSO bottle only for cell culture work; Open only in laminar flow hood

BEFORE STARTING

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

1 Gently detach cells from the tissue culture flask in the following manner:

1.1 Aspirate media

1.2 Wash with **2 mL** PBS, then aspirate.

1.3 Wash with **2 mL** trypsin.

1.4 Incubate for **00:05:00** at **37 °C** . 5m

2 Resuspend the cells in **10 mL** of DMEM with FBS. Aliquot into a centrifuge tube. Of course, if there is a T25 flask, resuspend in lesser volume.

3 Aliquot a small amount of the mixture (about **200 µl**) for cell counting. Ideally, cell viability should be in excess of 90% in order to achieve a good recovery after freezing. Keep at **-4 °C**

- We need 1 x 10^6 - 5 x 10^6 cells for freezing.

4 Centrifuge the cell suspensions at 1500rpm for **00:05:00** 5m

5 Discard supernatant carefully

6 Add about **3 mL** of freezing media into the tube and re-suspend.

6.1 Freezing media preparation:
- 10% DMSO, 20% FBS, 70% appropriate cell culture medium (with serum + antibiotics)
(e.g 100uL of DMSO + 200uL FBS + 700uL cell culture medium with FBS and antibiotics)

7 Dispense the aliquots of cell suspension into the cryogenic storage vials.

8 Cool the vials gradually as follows: 1h

-20 °C for **01:00:00**

-80 °C overnight

Liquid Nitrogen