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Freezing Adherent Cell Lines V.2

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Carolina Lopez[1]

[1]Washington University

Lopez Lab

Carolina Lopez
Washington University

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol describes how to freeze Adherent cells. Examples of these cells are: A549 cells, LLCMK2 cells, and MDCK cells.

Materials

FREEZING MEDIUM:

90% of TCM

10% of DMSO

TISSUE CULTURE MEDIUM (TCM): Filter through 0.2 µm filter

	Component	Amount	Conc. Supp.	Product information
	DMEM	500 mL		Gibco Cat. # 11965092 (#11965118-cs)
	Gentamicin	500 µL	50µg/mL	Gibco Cat #15750060 (#15750078-pk) – 50 mg/mL
	Sodium Pyruvate	5.0 mL	1mM	Corning Cat #25-000-Cl – 100mM
	L-Glutamine	5.5 mL	2 mM	Sigma Aldrich Cat #G7513 – 200mM
	FBS	50 mL	10%	

DMSO (Dimethyl sulfoxide): Sigma-Aldrich Cat# D2650

Freezing Adherent cells

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*Try to freeze cells at the lowest passage possible and confirm the cells are negative for mycoplasma before freezing.

1. Wash flask 2x with sterile 1X PBS.
2. Add 2mL of trypsin/T75. Incubate at 37ºC for 2-3 mins or until cells are detached from the flask.
3. Add 8mL of cell culture media and pipette up and down. Transfer all the media to a 15mL tube.
4. Centrifuge at 1200 rpm for 5 min.
5. Discard supernatant and resuspend cell pellet in 1mL of cell culture media. Add 9mL of media and pipette up and down to homogenize.
6. Count the cells. You will need your cell concentration/# for step 6.
7. Spin the tube containing the cells in media again at 1200 rpm for 5 min.
8. Meanwhile, prepare the freezing medium (see materials for recipe). Prepare the amount of freezing medium needed to dilute the cell pellet so that you have a final concentration of $1*10^6$ cells/mL.
9. Discard the supernatant and resuspend the pellet in 1mL of freezing medium. Add the remaining volume of the freezing medium. Make sure you homogenize the solution well.
10. Add 1mL of freezing medium containing $1*10^6$ cells/mL to cryotubes (caps screw from the outside). Label all the tubes with the following information:

- **Cell Name**
- **Generation/Passage**
- **Date**
- **Name**
- **Cell concentration (if there is space)**

11. Transfer the cells to a Mr. Frosty Freezing container containing isopropanol and then transfer the container to the -80ºC for 1 day. After 1 day, transfer the tubes to the liquid nitrogen. You can keep some vials in the -80ºC for a couple of months, however, the -80ºC is not for long-term storage.