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Thawing Frozen Adherent Cell Lines V.2

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

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Abstract

This protocol describes the procedures from thawing and expanding adherent cell lines

Materials

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%	

Mycoplasma Removal Agent (MRA) - MP Biomedicals. Cat # 093050044

Protocol

1 **Day 1**

1. Prepare media ahead of time and warm to room temperature
2. Spray down each bottle with 70% Ethanol before placing it into the TC hood
3. Pre-cool the centrifuge to 4ºC
4. Prepare and label a 6-well plate, and aliquot 9 mL of TCM into a 15 mL conical for each tube of cells to be thawed
5. Find the location of the cells you will be thawing in the liquid nitrogen inventory google doc
6. Transfer the cells between the liquid nitrogen and the TC room on ice
7. Record the information on the cryotube – date, number of cells, etc
8. Begin adding media from the 15mL conical to the still frozen cells, then transfer media back and forth between the two tubes until the cells have completely thawed and everything has been transferred to the 15 mL conical
9. Spin down the cells at 1200rpm for 10’ at 4ºC
10. Pour off the supernatant and break up the cell pellets by tapping the tubes
11. Add 1 mL of TCM to the cells, pipette up and down to break up any clumps, add 9 mL TCM, and transfer 1-2 mL of resuspended cells to a 6-well plate (cells should reach 80% confluency in two days)
12. Add 10 µl of Mycoplasma removal agent per 1 mL of media
13. Confirm the presence of cells in the well by microscope
14. Incubate at 37ºC

2 **Day 3** ***If cells tested negative for mycoplasma before freezing, only 1 round of MRA treatment is necessary. If mycoplasma presence is questionable or unknown 2 rounds of MRA treatment should be done – see mycoplasma protocol for more information.***

1. Warm media to room temperature
2. Remove media and wash cells once with PBS
3. Add 1 mL of 0.05% Trypsin-EDTA to wells and incubate plate at 37ºC for 1-3 minutes until cells are completely released from the plate
4. Add 4 mL of media to wells to halt trypsinization and transfer to a T25 flask
5. Incubate at 37ºC

3 **Day 4**

1. Warm media to room temperature
2. Check for cell adherence
3. If there are a lot of dead cells, change the media on the cells
4. If cells are nearing 50% confluence, trypsinize, transfer in 10 mL to a T75 flask – Label as passage #0