MAR 21, 2024

OPEN ACCESS

**DOI:**
dx.doi.org/10.17504/protocols.io.bp2l6xz3klqe/v1

**Protocol Citation:** Carolina Lopez 2024. Thawing Frozen Adherent Cell Lines. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bp2l6xz3klqe/v1



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

**Created:** Dec 17, 2023

# Thawing Frozen Adherent Cell Lines

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ABSTRACT

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

**Last Modified:** Mar 21, 2024

**PROTOCOL integer ID:** 92424

## Protocol (Sydney Faber_2023)

### 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 4C
4. Prepare and label a 6-well plate, and aliquot 9mL of media into a 15mL conical for each tube of cells to be thawed
5. Find the location of the cells you will be thawing in the -80C freezer inventory google doc
6. Transfer the cells between the freezer and the TC room on ice
7. Record the information on the cryotube – date, number of cell, 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 15ml conical
9. Spin down the cells at 1200rpm for 10’ at 4C
10. Pour off the supernatants and break up the cell pellets by tapping the tubes
11. Add 1mL of media per 2x10^6 cells to the cells, pipette up and down to break up any clumps, and transfer to the 6-well plate
12. Add 10ul of Mycoplasm removal agent per 1mL of media
13. Confirm the presence of cells in the well by microscope
14. Incubate at 37C

### 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 1mL of 0.05% Trypsin-EDTA to wells and incubate plate at 37C for 1-3 minutes until cells are completely released from the plate
4. Add 4mL of media to wells to halt trypsinization and transfer to a T25 flask
5. Incubate at 37C

### 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 10mL to a T75 flask – Label as passage #0