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

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Freezing Adherent Cell Lines

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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.

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Freezing Adherent cells

1

1. Wash flask 2x with sterile PBS.
2. Add 2mL of trypsin/T75. Incubate at 37 oC 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 medium again at 1200 rpm for 5 min.
8. Meanwhile, prepare the freezing medium. The amount of freezing medium to prepare should be the amount needed to dilute the cell pellet so that you have a final concentration of 1*106 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 the Stratagene chamber at 4C and then transfer the chamber to the -80C for 1 day. After 1 day, transfer the tubes to the liquid nitrogen. You can keep some vials in the -80C. These could last for at least 2-3 years.