Cell Culture

Cell Line Parameters

Each cell line is unique. The key parameters you should find out before working with a line are:

- Media and Growth Conditions
  - What base media, what concentration serum, and which additional inhibitors and antibiotics do you need to add?
  - What type of container will these cells need to be grown in?
- Splitting Conditons
  - How strongly adherent are your cells? Loosely adherent cells can use 0.05% Trypsin and incubate for 10 minutes, but more strongly adherent cells may need a higher concentration of Trypsin and longer incubation time.
  - How often must your cells be split? For how many passages are they stable?
  - When spinning down cells to remove debris, for how long and at what speed should you centrifuge them?

Defrosting Cells

To defrost cells from long-term-storage, follow these steps:

1. Prepare warm growth media.
2. Retrieve cells from freezer or collaborator.
3. Place cells in heat bath until edges of tube begin to thaw.
4. Add warm media drop-wise to tube.
5. Once tube is fully thawed, transfer its contents to 15 mL conical tube.
6. Bring tube volume up to 10 mL using warm media.
7. Spin down cells.
   - Label your plates during this step. Make sure to note the starting passage number.
8. Remove supernatant, resuspend cells in 10 mL warm media.
9. Measure cell count.
10. Add media, then cells to plate at desired concentration.
    - Use enough media to give 10 mL final concentration for 10 cm^2 dishes.
11. Shake plate left-right, then back-forth to distribute evenly on plate.

Measuring Cell Count

To count the number of cells in a solution, follow these steps:

1. Invert cell's tube to ensure even distribution of cells.
2. Get a new cell counting slide.
3. Spot 2 x 20 µL of cells into each of slide's holes.
   - There are four holes per slide, but you will only need to spot cells into two: one for each separated glass slip.
4. Place slide in cell reader.
5. Open up cell reader software on computer.
6. Click "Display image".
7. Ensure image is in focus.
8. Click "Count".
9. Check image to ensure the software correctly determined cell boundaries.
10. Repeat steps 4-9 for other side of slide.
11. Check duplicate readings are similar to one another.

Changing Media

Cells require a ready stream of new media to grow well. Most cells should have their media changed every 2-3 days, though you should regularly check the color of the media to ensure the pH has not dropped.

If their media is not red-orange, your cells have gone bad and you should throw them out. Do not try to save cells, a drop in pH will have applied selective pressure that can confound the results of your experiment.

To change cell media, follow these steps:

1. Aspirate off their media.
    - If you are using multiple cell lines or multiple conditions, use a new glass pipet tip for each aspiration.
2. Add new media to cells.
3. Bleach the vacuum pump line and check that the waste flask is clear in color.

Splitting Cells

As your cells grow, they will start to exceed the capacity of their plates. You will need to split the cells at this point into multiple containers. The growth rate of cells varies from cell line to cell line, but usually you will need to split them once a week.

To split cells, follow these steps:

1. Aspirate off media.
2. Optionally: Wash with PBS (without calcium or magnesium) to remove residual media components.
3. Add trypsin to cells.
4. Move cells to incubator and wait.
5. Check cells under microscope to ensure they are no longer adherent.
6. Transfer liquid containing trypsin and cells to a 15 mL conical tube.
7. Wash plate repeatedly with media or PBS and transfer to 15 mL conical tube.
    - Depending on the cell line, you may need to bump the plate to detach cells, wait longer, or wash them more thoroughly. Check your washed plates the first few times to ensure you have removed all of your cells. Being lazy at this step can lead to selection for less-adherent cells.
8. Spin down cells.
9. Aspirate off supernatant.
10. Resuspend in media
11. Measure cell count.
12. Add media, then cells to plate at desired concentration.
    - Use enough media to give 10 mL final concentration for 10 cm^2 dishes.
13. Shake plate left-right, then back-forth to distribute evenly on plate.

Freezing Cells

To freeze cells down, first follow the steps of lifting cells up and moving them into a 15 mL conical tube. Once you have cells in solution, follow these steps:

1. Close the conical tube and invert to distribute cells evenly in solution.
2. Take 2 x 20 µL of cells and measure their cell counts.
3. Spin down cells.
4. Aspirate off supernatant.
5. Resuspend cells in Freeze Media:
    - Freeze Media
        - 40% Media (RPMI, DMEM, etc)
        - 50% FBS
        - 10% DMSO
    - Use enough media to give 1e6 cells / mL.
6. Aliquot cells in 1 mL volume into 1.5 mL cryostorage (orange cap) tubes.
7. Put tubes in Mr. Frosty case.
    - Lightly tighten the cap to allow easy removal.
8. Store case in -80°C overnight.
9. Move tubes to cell storage freezer.
    - Located in cell culture stock room.