Culturing Frozen Cells

If you go to a lab to pick up the cells yourself, bring some dry ice in an ice bucket in which to transport the cells.

Background

Eukaryotic cells are usually frozen in medium with serum and a freezing additive, and are stored in liquid nitrogen at –196°C. If ordered commercially, they will arrive in a vial or ampoule on dry ice. Frozen cells must be thawed rapidly and cultured immediately to maximize viability. To remove the freezing additive the medium is changed after 24 hours.

Frozen cells should be cultured as soon as possible. If you cannot culture the cells immediately, store them in liquid nitrogen.

Materials

- Culture medium, warmed to 37°C
- Culture vessel
- 70% ethanol in a small beaker
- 1-ml and 10-ml pipet, pipettor and tips

CO_2 is used in an open culture system to regulate the pH of the cell medium. It is purchased as a cylinder of compressed gas and is dispensed into an incubator that can monitor and report the CO_2 content as well as maintain a set temperature.

Procedure

Cells are usually frozen in 1 ml volume, and are added to 10 ml of fresh medium.

1. Hold the vial in a 37°C water bath and agitate it rapidly. It will thaw in about 1 minute.
2. Drop the thawed vial in a beaker containing 70% ethanol and place it in a laminar flow hood for all manipulations.
3. Open the ampoule or tube. Do not allow ethanol to drip inside the vial.
4. Transfer contents of ampoule to culture vessel and immediately add warmed medium.
5. Replace cap and incubate for 24 hours.

*The freezing medium can also be removed **before** culture by spinning down the thawed cells and resuspending them in fresh medium. This is only necessary if the cells are particularly sensitive to the freezing additive or if you won't be there the next day to change the medium.*

6. Replace medium with fresh medium.
7. Incubate for 2–3 days or as indicated in the directions for those cells.

Ampoules are made of glass and are under pressure, and there is a very real chance that the ampoules can explode. Always open ampoules in a biosafety cabinet: If none is available, wear eye and face protection. You should wear eye and face protection whenever you are manipulating samples in liquid nitrogen.

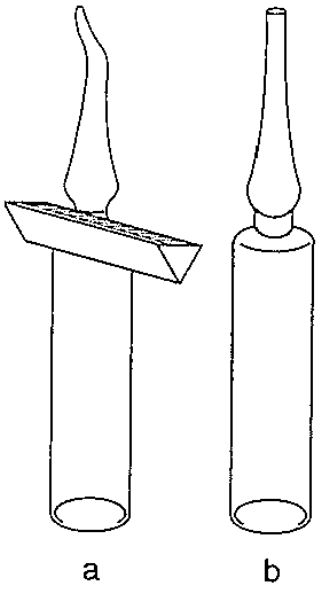

FIGURE 2.

Types of ampoules. Standard ampoules (*a*) must be nicked on the neck with a glass file that has been dipped in ethanol: After making a 1/8th score on one side, wrap the ampoule in gauze or a paper towel, hold the base firmly in the left hand and snap the top off with the right hand. Prescored ampoules (*b*) usually have a band around the neck, and can be wrapped and snapped open directly.

CELL MAINTENANCE

Routine Maintenance of Cell Lines

In order to maintain the cells, they must be:

- **Fed** (supplied with fresh medium). During growth, the medium will be depleted of needed factors and must be replaced.
- **Split** (passaged, the cell number reduced). During growth, the number of cells in the culture will increase beyond the capacity of the vessel and medium to sustain them.

Feeding and passaging are accomplished at the same time: It would be difficult to do one without the other.

- **Frozen.** Whenever you obtain or generate a cell line, aliquots of it must be frozen away. These aliquots are a backup for you should the cells phenotypically drift or all become contaminated.

PROTOCOL

Splitting Adherent Cells

1. Aspirate medium from the cell monolayer.
2. Gently add warm (37°C) PBS or culture medium without serum. Pipet the medium onto a wall of the culture vessel, not onto the cells, to avoid dislodging loosely adherent cells.
3. Aspirate the PBS or medium wash from the monolayer.
4. Add 0.25% trypsin/PBS, just enough to cover the cells when the culture vessel is tilted.

Cells are washed to remove traces of serum, which inhibits trypsin. You can use old medium without serum for washes.

5. Aspirate the trypsin, almost immediately: Leave it on the cells only 10–30 seconds before removing it.
6. Incubate the cells at room temperature for 5–15 minutes, checking every couple of minutes to see whether the entire monolayer slides when you tilt the culture vessel. (You can see this macroscopically.) When it does, you can end the incubation. Alternatively, you could incubate the cells at 37°C for 5 minutes.
7. Add fresh medium (the same volume you removed) and pipet vigorously to break cell clumps. Check on the inverted scope to be sure you have a single cell suspension and pipet until you do.
8. Remove 1 ml of cells to a microfuge tube.
9. Count the cells.
10. Figure out the dilution you must make to get to the recommended seeding concentration.
11. Remove that quantity of cells to a fresh culture flask.
12. Add the calculated amount of fresh medium (which has been warmed to 37°C).