

General procedures for maintenance of *Drosophila* cell lines

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This protocol is intended to offer general guidelines for DGRC users who are not experienced with *Drosophila* cell culture. Specific information on a variety of topics is provided in separate protocols and on the webpages for specific cell lines is available from the DGRC website (<http://dgrc.cgb.indiana.edu/>). General guidelines for sterile technique are available from a variety of published sources.

What to do with your cells when they arrive

We usually ship cells as frozen ampoules; these ampoules should be thawed and the cells placed in fresh medium as soon as they arrive; see the separate protocol on thawing cells for detailed instructions on their recovery. **Do NOT place the frozen ampoules in a -80 freezer or in liquid nitrogen when they arrive; the rapid change in temperature often causes substantial damage to the cells.**

In some circumstances, we ship cells as a growing culture. In that case, you will receive a 25 cm² T flask with approximately 50 ml of culture, nearly filling the flask. This is a much larger volume than would normally be used in this flask (see below); the intent is to minimize mechanical damage from splashing during the shipment. Once the cells arrive, you should proceed as follows:

- Place the flask in its normal orientation (cap on the side), and allow the cells to settle onto the bottom of the flask. Look at the cells in an inverted microscope to assess their density.
- If the bottom of the flask is covered with a dense layer of cells, then gently resuspend the cells (using a serological pipet and/or a Pasteur pipet) in the medium that is in the flask, and dispense the resulting cell suspension into fresh plates and/or T-flasks (see below for volumes). You should have enough cells suspension to make 5 100 mm plates. Put the cultures into the incubator, and let them grow.
- If there are not enough cells to cover the bottom of the flask, then gently withdraw all but about 5 ml of medium. Put the flask into the incubator, and let the cells grow.
- There will be some damage to the cells as a result of their travel. Expect to see some dead cells, and some delay in reaching their normal growth rate, but dividing cells should be visible within a day or two.



Major differences between mammalian cell culture and *Drosophila* cell culture

Those who are trained in mammalian cell culture should be aware of several major differences in the techniques used for mammalian and *Drosophila* cell lines:

- Temperature of culture: *Drosophila* cell lines should be grown at 25° C., unless otherwise indicated. Some laboratories have been successful growing *Drosophila* cells at room temperature, but this is inadvisable if there are large fluctuations in room temperature. Ideally, use a refrigerated incubator set at 25°.
- Gas phase: *Drosophila* culture media do not use CO₂ for buffering; hence the gas phase for *Drosophila* tissue culture should be ordinary air. In order to prevent excessive evaporation, we advise keeping plates in sealed food-storage containers (e.g. Tupperware or Rubbermaid) inside the incubator.
- Removal of cells from the surface: For most cell lines we recommend simply blowing medium at the cells from a serological pipet or (for more strongly adherent cells) from a Pasteur pipet. A reasonably dense layer of cells adhering to the surface of a plate looks to the naked eye like a surface scum; it is generally easy to monitor the removal of the cells from the surface simply by watching the disappearance of the “scum”. It is also often helpful to monitor the process by occasionally checking the plate in an inverted microscope. The most commonly used *Drosophila* cell lines (Kc, S2) adhere very loosely to the substrate, and require little or no effort to dislodge them. Some lines adhere strongly to the substrate and cannot be readily removed by blowing medium at the cells; some of these lines can be trypsinized at each transfer (see separate protocol), or loosened with a cell scraper. See notes on the individual cell line webpages for specific suggestions.
- “Contact inhibition”: I have never seen a *Drosophila* line that shows the classic features of contact inhibition. Most *Drosophila* lines continue to proliferate after they have covered the substrate; they either pile up to form more than a single layer, or they come off the surface and grow in suspension.

General procedures

In general, *Drosophila* lines grow exponentially in the range 10⁶ – 10⁷ cells/ml. At 10⁷ cells/ml, the cells completely cover the surface, if they are maintained in the volumes recommended below. Robust lines (e.g. Kc, S2) can simply be diluted 10-fold when they reach 10⁷/ml. More delicate lines (e.g. most imaginal disc and CNS lines) should be diluted less (generally 2-3 fold), because they tend to be sensitive to over-dilution, it is often hard to estimate their concentration accurately, and there are often significant losses because of cell breakage during the transfer.

We strongly recommend that you prepare a few frozen ampoules as backup as soon as the cells are growing well. A protocol for this procedure is available from the DGRC website. Although *Drosophila* cells are in general more stable in their properties than mammalian lines, they do



accumulate mutations over time, and it is therefore a good idea to go back to the frozen stock every few months. Frozen cells degrade slowly at -80° (the rate varies with the cell line); they are essentially infinitely stable in liquid nitrogen, but if a box of ampoules is repeatedly removed from liquid nitrogen to retrieve individual ampoules for use, the remaining ampoules do degrade.

Vessels for growing cells

We use tissue culture-grade Petri plates, 100 mm diameter, for routine maintenance of cell lines. T flasks work equally well, but we find that the cells are much more easily accessible in plates than in the more expensive T flasks. T flasks can be sealed, and are therefore convenient when a culture is expected to take a long time before it is ready to be expanded; we therefore recommend T flasks when thawing frozen cells (see separate protocol for thawing cells). Do NOT use bacteriological-grade Petri plates.

The volume of culture in a given vessel is determined by the surface area; the medium should be about 2-3 mm deep, to avoid excessive evaporation while permitting adequate oxygen exchange. The volumes that we recommend for various tissue culture vessels are:

100 mm Petri plate:	10 ml
60 mm Petri plate:	4 ml
35 mm Petri plate:	1 ml
75 cm ² T flask:	15 ml
25 cm ² T flask:	5 ml
6-well plate:	2 ml/well
12-well plate:	1 ml/well
24-well plate:	0.5 ml/well (seal outside of plate with Parafilm to minimize evaporation)
48-well plate:	0.3 ml/well (seal outside of plate with Parafilm)
96-well plate:	0.1 ml/well (seal outside of plate with Parafilm)

The standard embryonic lines (e.g. Kc, S2, S3) can also be grown in spinner flasks; this is a convenient way to grow liters of cells. The volume of medium should be sufficient to cover the magnetic stirrer, but should be no more than half the manufacturer's recommended volume; larger volumes produce very poor growth, presumably because of insufficient oxygen. We have not tried to grow other lines in spinner flasks, but we would predict that the more delicate and more surface-adherent lines would do poorly.

Antibiotics

We do not add antibiotics to the medium; we have found it to be unnecessary if one uses good sterile technique. If you wish to use penicillin and streptomycin, feel free to do so; it is not harmful.

