Cell Line Shipping Instructions

The following technical note is offered to give guidance as to the preferred method for isolating, packaging and shipping live and frozen cell lines to preserve DNA quality when transporting. The media and growing conditions described in this Tech Note are to be used when no other media recipes or growing conditions are available. Established conditions for your cell lines are to take precedence over the information provided here. Packaging materials must be leak-proof and meet the general requirements of the US Postal Service and other carriers, and customs authorities where applicable.

Shipping Instructions for Live Cell Lines

- Email recipient to provide information on shipping cells, with the expected arrival day (from Tuesday to Friday).
- Seed cells into one T25 flask.
- Ship the cells on day 2 or 3 after passage, or seed the culture with 600,000 to 800,000 cells. The cultures must be actively dividing (in log phase).
- Prior to shipping, fill the T25 flask to capacity with warmed media, tighten the cap and seal it with Parafilm. (Use plug-seal caps only; do not use vented caps).
- Print the shipping form and fill out. Place form in a sealed bag and include in shipment container.
- Wrap the T25 flask and media tube in absorbent paper towels and place them in separate sealed plastic bags. Wrap each bag in bubble wrap.
- Place culture flasks and shipping forms in a small polystyrene box and fill with bubble wrap or packing material so that the contents will not move during shipping. In colder climates, addition of temperature control packaging (e.g. Saf-T-Pak Phase Control Material) can stabilize the culture temperature during transit.
- Place the polystyrene box inside a slightly larger cardboard box and seal with packing tape. SHIP AT ROOM TEMPERATURE. Do not ship on ice packs or cold packs.

Send the package to recipient by next-day delivery service (FedEx Priority Overnight or UPS Next Day Air) for delivery from Tuesday to Friday; avoid shipments arriving on weekends or holidays. If shipping internationally, ensure that samples are admissible and that proper declarations are made with customs authorities. Email recipient with the tracking number and the shipment delivery date.
Shipping Instructions for **Frozen** Cell Lines

- Prepare cells according to the provided Freezing Cells protocol (next page).

- For frozen cell lines, aliquot in amounts of 2 million cells.

- Place cryovials inside a 50 ml conical tube. Print the shipping form and fill it out. Place form in a sealed plastic bag and include it in the shipment container.

- Ship the materials in a polystyrene box (with at least a 1” thick walls and minimal inside dimensions of 8” x 6” x 4”), which is filled at least halfway with dry ice.

- Apply Dry Ice labels on outside box ([UN 1845](https://www.联合国.org/1845)).

- Send the package by next-day delivery service (FedEx Priority Overnight or UPS Next Day Air). If shipping internationally, ensure that samples are admissible and that proper declarations are made with customs authorities. Accommodate for customs inspection accordingly. We recommend World Courier or another courier that will replenish dry ice during transit and while waiting in customs. Email recipient with the tracking number and the shipment delivery date.

Shipments should only be delivered from Tuesday to Friday; avoid shipments arriving on Saturdays, Sundays, or national holidays.
Preparation for Sending **Frozen** Cells

The following should only be used when no other media or culture conditions are available.

**Recommended Media**

1. RPMI 1640 (Sigma, p/n R8758).
2. 2mM L-Glutamine, 100X (BioWhitaker, p/n 17-905C).
3. 15% FBS (Gibco, p/n 10438-026 or BioWhittaker, p/n 14-503F).
4. [Add Pen/Strep to inhibit contamination, 100X (Gibco, p/n 15140-148)].

**Recommended Culture Conditions**

1. RPT25 flask with 10-20 mL media (20 mL maximum).
2. Place flask in upright position.
3. 37°C under 5% CO₂.

**Passaging Cells**

1. Density
   a. Do not let cells reach maximum density otherwise slow growth may result.
   b. Split cells so there is no less than 200,000 viable cells/mL.
2. Reagents
   a. Write down lot # for serum and medium.
   b. FBS
      i. Range of FBS is 5-15%.
      ii. Can grow most lymphoblast cell lines in heat inactivated FBS.
         1. If cells grow poorly, inactivate FBS by placing at 56°C for 30 min.
3. Procedure
   a. Warm complete media to 37°C before adding to cells.
   b. Use pipet to gently break up cell aggregates before counting.
   c. Split cells every 3-4 days.

**Freezing Cells**

1. Grow up 20 million cells for each cell line by pooling flasks (if necessary).
2. Calculate total number of viable cells; pipette first to gently break up cell aggregates.
3. Centrifuge for 10 min at 120 × g (860 rpm in GH 3.8A rotor) at 4°C.
4. Resuspend pellet in freezing media at 5x 10⁶ cells/mL:
   a. Freezing media
      i. RPMI 1640
      ii. 20% FBS
      iii. 6% DMSO
5. Make 1 mL aliquots in cryovials.
6. Freeze overnight at -1°C/min in -80°C freezer using freezing chamber containing fresh isopropanol.
7. Transfer cryovials to liquid nitrogen tank for storage.
Shipping Form (please fill out and ship it with the sample)

If the sample is cell line, is it a primary cell line?  Yes  No  (circle one)

Cell line type:  Suspension  Adherent  (circle one)

Passage #

Titer:

Doubling time (in days):

Recommended Culturing Conditions:

Split ratio:

Temperature:

Percent CO₂:

Medium:

Serum:

Substrate:

Sub-cultivation method:

Antibiotics:

Comments: