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

- Cells may be frozen either as pellets, or as a suspension in freezing media (Pages 3 and 4).
- For frozen cell lines, aliquot in amounts of at least 1.5 million cells. Although only one aliquot is required for ultra-high molecular weight DNA extraction, it is recommended to send a second aliquot as a backup.
    - Note: In order to ensure sufficient yield and maximum quality of DNA, it is highly recommended to count cells **before** freezing, using a hemocytometer or some other cell counting device.
- 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).
- 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.

Preparing **Frozen** Cell Pellets

Recommended Buffer

1. Prepare Stabilizer Buffer by combining 49 µl of Bionano Cell Buffer + 1 µl Bionano DNA Stabilizer for each of the pellets you plan to prepare.
    - **Note:** If these reagents are not available, it is recommended to prepare frozen cells in freezing media (next page). Otherwise, PBS (without Ca^{+2} or Mg^{+2}) may be used instead of Bionano Cell Buffer + Stabilizer, but DNA molecule length may suffer.

Counting Cells

2. Pipet isolated cells in growth media repeatedly to ensure a uniform suspension when sampling.
3. Count viable cells with a cell counting device.
    - **Note:** If possible, make sure the cells are actively growing with high viability as this maximizes quality and size of isolated gDNA.
4. Calculate the volume of original cell stock required for 1.5 x 10^6 cells.
5. After pipet mixing to ensure a uniform suspension, transfer volume for 1.5 x 10^6 cells to a labeled 15 mL polypropylene conical vial.

Freezing Cells

6. Pellet the cells by centrifugation using a swinging bucket rotor at 2,200 x g for 2 minutes at room temperature.
7. Remove the supernatants by decanting into a waste container with bleach and use a Kimwipe® to absorb residual liquid from inverted cell pellet conical vial.
8. Add 40 µl of Stabilizer Buffer or PBS on top of each pellet.
9. Disrupt the pellet with a 200 µl wide bore tip, then continue to resuspend the pellet by pipetting up and down 10 times.
10. Transfer the entire volume of suspension (>40 µl) into a labeled 1.5 mL microcentrifuge tube with a standard 200 µl tip.
11. Pellet the cells in a microcentrifuge by spinning at 2,200 x g for 2 minutes at room temperature.
12. Using a standard 200 µl tip, carefully remove as much of the supernatant as possible without disturbing the pellet.
13. Freeze and store the cell pellets at -80°C.

Preparing **Frozen** Cells in Freezing Media

The following protocol has been tested with the human lymphoblastoid cell line GM12878, and is provided as an example. If an existing protocol has been optimized to maintain high viability in your particular cell lines, we recommend that you continue to use that protocol.

Example 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)].

Example Culture Conditions

1. RPT25 flask with 10-20 mL media (20 mL maximum).
2. Place flask in upright position.
3. 37°C under 5% CO_2.

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 enough cells to seed/maintain a backup culture at your facility after removing 3 million viable cells for shipment. Flasks may be pooled 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 1.5x 10^6 cells/mL:
    a. Example 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 for Cell Lines

(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 (cells/mL):

Doubling time (in days):

Recommended Culturing Conditions:

Split ratio:

Temperature:

Percent CO_2:

Medium:

Serum:

Substrate:

Sub-cultivation method:

Antibiotics:

Comments:

Bionano Genomics
9540 Towne Centre Drive, Suite 100
San Diego, CA 92121