RUNTOGEN

Important technical information for successful culture of your cells

Upon Cells Arrival and Basic Troubleshooting Tips

Please read before handling cell cultures

Important: This document is not applicable for induced Pluripotent Stem Cells (iPSCs) a separate document is available for iPSCs on each of the iPSC product detail pages

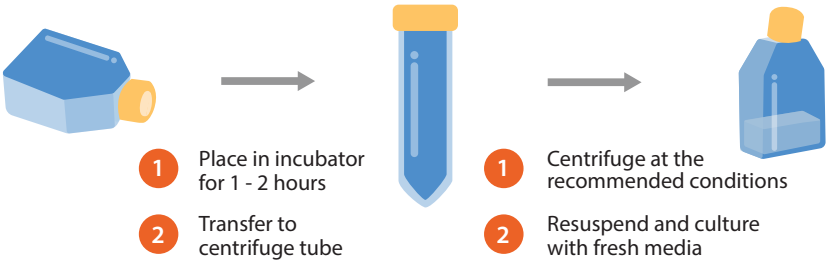
Live Culture Flask Transported at Room Temperature

1. Upon receiving cells, ensure all label information matches the shipping list. Then, examine the flask for signs of breakage, leakage, and contamination. If any abnormalities are observed, reach out to o ur technical team (info@runtogen.com) with your order number, cell line catalog, photos, and a det ailed description of your observations.
2. Disinfect the neck and cap area of the cell culture flask with 75% isopropanol and place in an incub ator for 1-2 hours. Then, while working in a biosafety cabinet:

For adherent cells: if there is an excess amount of culture medium, remove the extra and leave 3-4 ml in the flask. Then, incubate cells at the recommended conditions overnight.

For suspension cells: if there is an excess amount of culture medium (more than 5.0ml), collect the entire contents of the culture flask, centrifuge at the recommended conditions, and discard the supernatant. Resuspend the cell pellet in pre-heated complete growth medium and seed cells into an appropriate culture vessel, as per experimental requirements.

3. On the following day, check cell health and confluency by viewing cells under a microscope. If the culture is healthy, return the flask to the incubator until subculturing is required.



4. For subculturing instructions, refer to the product datasheet.

Frozen Cryovials Transported on Dry Ice

1. Upon receiving cells, ensure all label information matches the shipping list. Then, examine the vial for signs of breakage or leakage. If any abnormalities are observed, reach out to our technical team (info@runtogen.com) with your order number, cell line catalog, photos, and a detailed description of your observations.
2. Immediately store frozen vial(s) at a temperature below -130°C, preferably in liquid nitrogen vapor phase storage.
3. For cell culture guidelines, refer to the product datasheet.

Cell Culture Troubleshooting Guide

1. **Adherent Cells Not Attaching:** Some adherent cells require pre-treatment of culture vessel with special coatings to facilitate adhesion. Coated culture vessels should be prepared prior to thawing and/or passaging cells. Cells sensitive to Trypsin-EDTA require gentle enzymatic digestion. Any enzymatic digestion process should be neutralized using serum containing complete medium.
2. **Dirty Cell Background:** The empty areas between cells constitute the cell background. If moving, similar size black dots are observed, this may be a sign of mycoplasma contamination; cells can be treated with mycoplasma decontamination agent as described below. If black dots of non-uniform size and shape are observed, they are most likely cell membrane fragments released after cell rupture. These can be typically removed by changing the media.
3. **Mycoplasma Contamination:** Treat cells with Mycoplasma Elimination Cocktail, where applicable.
4. **Bacterial Contamination:** This contamination results in a very cloudy culture medium and appears as sand-like particles capable of movement, under the microscope. Bacterial contamination proliferates rapidly.
5. **Yeast Contamination:** Yeast appears as rod-shaped particles or chains of two to four or more particles and can sometimes be multi-branched, without movement. Their proliferation rate is slower than bacteria, but a significant outbreak may occur. Use common antimycotic agents suc h as Amphotericin B and mycostatin (Nystatin) to treat cells.
6. **Fungus Contamination:** Fungus appears as thin filamentous mycelia and is visible to the naked eye. Fungus is usually slow growing and can be treated with Amphotericin B.

For technical assistance, please retain microscopic images of the cells and contact
info@runtogen.com