CellBiologics
A CELL ABOVE THE REST

For Research Use Only

Primary Cell Culture Protocol

- All cell culture procedures must be performed in a biosafety cabinet.
- All culture media, supplements, and reagents must be sterile filtered through a 0.2 μm filter.
- Use aseptic technique to prevent microbial contamination.
- Upon receipt of packaging, cryopreserved cells should be promptly transferred from the dry ice shipping container to a liquid nitrogen storage tank.

Medium

- For information on suitable complete culture media (e.g., serum and other supplements), visit the Cell Biologics website www.cellbiologics.com.
- Prewarm only the cell culture medium you will be using (37°C).
- When recovering frozen cells, changing medium, or splitting cells, prewarm 5 ml of culture medium for a T25 flask and 8-10 ml of culture medium for a T75 flask.
- Repeated prewarming of complete culture medium is not recommended.

Coating of flasks or culture dishes

- Coat sterile culture dishes or flasks with gelatin-based coating solution (Cell Biologics, Catalog No. CB6950) for 2 minutes, then aspirate excess solution before seeding cells.

Live Cell Arrival Handling

- Upon receipt of live cells in T25 or T75 flasks, remove the sticker from the filter cap and place the flask with 6-20 ml of existing culture medium in a 37°C, 5% CO_2 incubator for 1 hour.
- Replace with desired Cell Biologics cell culture medium pre-warmed to 37°C.
- These cells can be subcultured at a 1:2 ratio at 90% confluency or frozen at 95-100% confluency for future use.

Cell Recovery from Cryovial

1. Thaw cells quickly by placing the vial in a 37°C water bath for less than 1 minute or until only a few ice pieces remain in the vial.
2. Immediately remove the vial and wipe it with 70% ethanol.
3. Transfer cells from the vial to a sterile 15 ml conical centrifuge tube containing 5 ml of pre-warmed Cell Biologics cell culture medium.
4. Rinse the vial with an additional 0.5-1 ml of medium to collect any remaining cells in the vial.
5. Centrifuge the cells at 200 x g for 5 minutes. Note: Centrifugation of cells after thawing to remove residual DMSO is recommended.
6. Carefully aspirate the supernatant and resuspend the cell pellet in 5 ml of Cell Biologics Cell Culture Growth Medium. **Add the resuspended cells to a T25 flask** pre-coated with gelatin-based coating solution (Cell Biologics, Cat. No. CB6950).
7. Place the inoculated T25 flask in a 37°C, 5% CO_2 humidity incubator and culture for 12-16 hours.
8. Change the medium on the second day and every 24 hours thereafter to remove non-adherent cells and replenish nutrients. Note that the medium should be changed daily (5mL for a T25 flask and 10mL for a T75 flask).
9. Cells should be checked daily under a microscope for confluence and proper cell morphology.

Primary Cell Culture Expansion

1. Remove the culture medium and wash the cells twice with room temperature 1X phosphate-buffered saline (without calcium and magnesium).

Cell Biologics Company - 2201 West Campbell Park Drive, Chicago, IL 60612
website: http://www.cellbiologics.com - e-mail: service@cellbiologics.com - Phone: 1-312-226-8198

2. Incubate the cells with 0.25% Trypsin-EDTA solution (Cell Biologics, Cat. No. CB6914) prewarmed to 37°C for 3-5 minutes. Use 2.0 ml of Trypsin-EDTA solution for a T75 flask and 0.5-1 ml for a T25 flask.
3. Once the cells have detached (**a few firm taps on the flask may help**), immediately add 5-10 ml of Cell Biologics cell culture medium (with fetal bovine serum to neutralize the trypsin) to the flask and gently pipette up and down several times.
   Note: We recommend splitting primary cells at the following ratios:
   Primary cells grown to confluence in a T75 flask can be split into two T75 flasks (10 ml of medium per T75 flask).
   Primary cells grown to confluence in a T25 flask can be split into two T25 flasks (5 ml of medium per T25 flask).
4. Seed the cells into fresh flasks/plates pre-coated with gelatin and return the flasks/plates to a humidified 37°C, 5% CO_2 incubator.
5. Change the medium on the second day and every 24 hours thereafter to remove non-adherent cells and replenish nutrients. Before each medium change, pre-wash the cells twice with 1X PBS (without calcium and magnesium).
6. Cells should be checked daily under a microscope for cell confluence and appropriate cell morphology.

We recommend splitting primary cells at the following ratios
- The recommended split ratio for primary cells is 1:2.

Cell Freezing Procedure
Materials:
1. 1X Phosphate-Buffered Saline (PBS-1X)
2. 0.25% Trypsin-EDTA Solution (Cell Biologics, Cat. No. CB6914)
3. Tissue Culture Medium
4. Freezing Medium (10% DMSO, 50% FBS, and 40% culture medium, Cell Biologics, Cat. No. CB6916)
5. Labeled Cryovials
6. Confluent Cells

----

1. Remove and discard the cell culture medium from the culture flask.
2. Rinse the adherent layer 2-3 times with 10 ml of sterile PBS (1x, calcium- and magnesium-free) to remove any residual culture medium.
3. Incubate the cells in 0.25% Trypsin-EDTA solution (Cell Biologics, Cat. No. CB6914) prewarmed to 37°C for 3-5 minutes (use 2.0-3.0 ml of Trypsin-EDTA solution for a T75 flask and 0.5-1.0 ml for a T25 flask). Once the cells have detached **(a few firm taps on the flask may help**), immediately add 10 ml of Cell Biologics cell culture medium (with fetal bovine serum to neutralize the trypsin) and gently pipette up and down 1-2 times.
4. Transfer the cell suspension to a centrifuge tube.
5. Centrifuge the cells at 200 x g for 5 minutes.
6. Carefully remove the supernatant using a sterile Pasteur pipette.
7. Add 1 ml of freezing buffer (CB6916) to each vial and quickly resuspend the cell pellet.
8. Place the cells/vial in a Nalgene "Mr. Frosty" freezing container filled with 100% isopropanol and freeze at -80°C for 6-12 hours.
9. Transfer the cells/vials to a liquid nitrogen tank and store indefinitely.

We recommend freezing primary cells at the following ratios
- Confluent primary cells grown in a T75 flask can be frozen in 2 cryovials.
- Confluent primary cells grown in a T25 flask can be frozen in 1 cryovial.

Note:
1. Once cells reach confluence, you can allow them to overgrow for 24 hours before performing any cell-based assays, staining, FACS analysis, or designed experiments (change the medium daily and pre-wash the cells 2 times with 1X PBS between medium changes).
2. Before performing an experiment, filter the cells using a 40 µm cell strainer (BD 352340) to remove any cell clumps and most dead/floating large cells.
3. If you have any questions about your cultured cells, please send us light microscopy images of your cells (cell confluence >70-80%).