Cell Culture Protocol

Cell culture and subculture of Human Mesenchymal Stem Cells & Primary Cells manufactured at Vitro Biopharma

Applicable Products: SC00A1, SC00AD1, SC00BM1, SC00P1, SC00A5, CAF01, CAF02, CAF03, CAF04, CAF05, C05DON, CAF06, CAF07-AD, C07ADDON, CAF07-S, CAF08, CAF09, and CAF10.

Media selection is dependent on the cell culture. Vitro Biopharma offers a variety of cell products with medium that is optimized for growth and continuous passage. Please contact our Technical Support team at info@vitrobiopharma.com for any additional information regarding this protocol.

- All Mesenchymal Stem Cells (MSCs) should be cultured in MSC-GRO™ medium; Low Serum Complete (Cat. No. SC00B1), Serum-Free and Xeno-Free (Cat. No. PC00B2), or Serum-Free (Cat. No. PC00B3).
- All Primary Cell Lines and Cancer Associated Fibroblasts (CAFs) with the exception of CAF08 should be cultured in MSC-GRO™ VitroPlus III Low Serum Complete Medium (Cat. No. PC00B1).
- Pancreatic Stellate CAFs (Cat. No. CAF08) should be cultured in MSC-GRO™ Pancreatic Stellate CAFs Maintenance Medium (Cat. No. PC00B5).

Establishing Cultures From Cryopreserved Cells

Use of these cell lines requires prior experience in standard methods of mammalian cell culture. In particular, sterile technique is required in a dedicated cell culture facility that is free from contamination. Cryopreserved cells may be used to establish cultures immediately upon receipt or they may be stored for use at a later time. If stored, it is preferable to store in the vapor phase of liquid N_2.

To establish cultures from cryopreserved cells, first ensure that adequate equipment and reagents are available to perform the necessary procedures in a timely manner. These cells require culture in a 37°C, CO_2 cell culture incubator, calibrated to 5% CO_2. If the capabilities are available, we highly recommend growing the cells in hypoxic conditions. Mesenchymal stem cells exist in hypoxic environments within the body, and we have found accelerated growth rates under reduced oxygen conditions (1 to 5% O_2). However, these cells can be cultured at ambient oxygen levels (~20% O_2) which results in reduced growth rates and passages. We highly recommend using Modular Incubation Chambers (MICs) that may be purchased through Billups-Rothenburg, Cat. No. MIC-101. Please contact technical services for information about various equipment and instrumentation options available to establish reduced oxygen cultures.

Inoculation from Cryopreservation

Thawing cells from cryopreservation for inoculation requires a water bath equilibrated to 37°C. Exposure of closed vials containing liquid nitrogen to a 37°C water bath is an **explosion hazard**. Please follow your institutions guidelines for safe handling of cryogenically preserved cells. Provide continuous, gentle agitation (swirling), to the vial while it is submerged in the 37°C water bath. Continue with agitation until the cells are completely thawed and no ice remains within the cell suspension, usually about 1 to 2 minutes. Maximum cell viability is dependent on rapid and complete thawing of frozen cells.

We recommend performing a Post-Thaw Viability and Cell Count prior to inoculation using an automated cell counter. For example, create a 1:5 dilution with the cells being the sample and 1x PBS or equivalent (basal medium is acceptable), being the diluent.

- When working with 250µL total in a microcentrifuge tube, add 50µL cells to 200µL 1x PBS

Once the cell count has been established, plating density can then be determined. For optimal plating density with Vitro Biopharma's cell lines, plate the cells at 5,000 to 10,000 cells/cm^2 in the desired, tissue culture treated flask(s)/plate(s). We recommend direct inoculation of cultures from the cryopreservation media after completion of the post-thaw viability and cell count. Washout of the cryopreservation medium directly after thawing has been shown to decrease viability.

Plating

We provide MSC-GRO™ media for optimal proliferation of MSCs/CAFs. Data that is presented on our website (www.vitrobiopharma.com) shows improved growth, viability, and doubling time.

Add the appropriate volume of culture medium to the plate or flask to be used for culture. We recommend adding 5mL of medium per T12.5, 10 mL of medium per T25, and 20mL of medium per T75. Use these guidelines to determine the appropriate volume of medium for your application. Following inoculation with the desired volume of cell suspension, gently agitate the flask or plate to ensure homogeneous distribution of the cells within the cell culture medium. Allow cultures to incubate in 1-5% O_2, 5% CO_2, with a N_2 balance at 37^{o}C in a humidified environment. Monitor cell growth by visual inspection. When cultures become 80-90% confluent, proceed to subculture. This should require about 3 to 4 days of continuous culture, but this time depends on several factors.

Subculturing

1. Once the cells are >80% confluent, wash the flask(s) 1-3 times with PBS (5mL for T12.5, 10mL for T25, and 20mL for T75).
2. After washing, add Accutase (2.5-3mL for T12.5, 5mL for T25, 10mL for T75) and incubate on orbital shaker with moderate agitation at 37^{o}C for 15 minutes.

   NOTE: Alternatively, trypsin or an equivalent may be used for dissociation but may result in decreased viability, decreased passage capability, and decreased overall cell health. We do not recommend this method.

3. Visualize the culture. If necessary, assure complete detachment of cells as by tapping the flask or plate firmly on a solid, flat surface.
4. Transfer the dissociated cells from the flask to a centrifuge tube. Wash the flask with an equivalent amount of 1x PBS and combine the wash with the dissociated cells in the centrifuge tube.
5. Centrifuge the cell collection at 1500RPM for 10 minutes at 4˚C. A visible cell pellet should form at the bottom of the centrifuge tube.
6. Transfer the centrifuge tube back into the biological safety cabinet. Then aspirate and discard the supernatant. Ensure to NOT disturb the cell pellet when aspirating. If the pellet is disturbed, recentrifuged.
7. Resuspend the cell pellet in 1mL 1x PBS by repetitive elutriation to create a single cell suspension.
8. Count the cells using an automated cell counter or hemacytometer. We recommend creating 1:20, 1:10, and 1:5 dilutions read in triplicate with the diluent being 1x PBS.
9. For routine passage, we recommend plating the cells at 5,000 to 10,000 cells/cm^2 in the desired cell culture flask(s)/plate(s).
   Additional Notes:

- For optimal viability, complete the subculture process within 2 hours or less of dissociation.
- Fully adapted MSCs/CAFs typically require about 4 to 5 days to reach about 90% confluence, although this is dependent on several different factors.
- We recommend feeding every 3-5 days.
- For longer or shorter periods between subculture, cultures may be inoculated at lower or higher densities.
- Our suggested procedures are provided as guidelines and may require adjustments within different laboratory environments.

Technical Service

For any additional questions or concerns, please contact Vitro Biopharma at 303-999-2131 and ask to speak with technical service or please email us at info@vitrobiopharma.com

We are here to help in your cell culturing process, and we thank you for your business!