

Products

Products applicable to this Cell Culture Protocol.

Mesenchymal Stem Cells (MSCs)

SC00A1	Human Umbilical Cord Derived Mesenchymal Stem Cells (MSCs)
SC00AD1	Human Adipose Derived Mesenchymal Stem Cells (MSCs)
SC00BM1	Human Bone Marrow Derived Mesenchymal Stem Cells (MSCs)
SC00P1	Human Placenta Derived Mesenchymal Stem Cells (MSCs)

Cancer-Associated Fibroblasts (CAFs)

CAF01	Human Endometrial Adenocarcinoma Cancer-Associated Fibroblasts (CAFs)
CAF02	Human Ovarian Carcinoma Cancer-Associated Fibroblasts (CAFs)
CAF03	Human Glioblastoma Cancer-Associated Fibroblasts (CAFs)
CAF04	Human Renal Cell Carcinoma Cancer-Associated Fibroblasts (CAFs)
CAF05	Human Colorectal Adenocarcinoma Cancer-Associated Fibroblasts (CAFs)
CAF06	Human Breast Carcinoma Cancer-Associated Fibroblasts (CAFs)
CAF07-AD	Human Lung Adenocarcinoma Cancer-Associated Fibroblasts (CAFs)
CAF07-S	Human Lung Squamous Cell Carcinoma Cancer-Associated Fibroblasts (CAFs)
CAF08	Human Pancreatic Adenocarcinoma Cancer-Associated Fibroblasts (CAFs)
CAF09	Human Melanoma Cancer-Associated Fibroblasts (CAFs)
CAF10	Human Prostate Carcinoma Cancer-Associated Fibroblasts (CAFs)
CAF11	Human Gastric Carcinoma Cancer-Associated Fibroblasts (CAFs)
CAF12	Human Head and Neck Squamous Cell Carcinoma Cancer-Associated Fibroblasts (CAFs)

Normal Fibroblasts

C05DON	Human Colorectal Fibroblasts
C07ADDON	Human Lung Fibroblasts
SC00A5	Human Pancreatic Fibroblasts

Media

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

Mesenchymal Stem Cells (MSCs) should be cultured in:

SC00B1	MSC-GRO™ Low-Serum Complete Mesenchymal Stem Cell (MSC) Medium: 500mL
PC00B2	MSC-GRO™ VitroPlus III, Serum-Free, Xeno-Free Complete Medium: 500mL

Normal Fibroblasts & Cancer Associated Fibroblasts (CAFs) should be cultured in: * Excluding CAF08*

PC00B1	MSC-GRO™ VitroPlus III, Low-Serum Complete Mesenchymal Stem Cell (MSC) Medium: 500mL
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CAF08 should be cultured in:

PC00B5	MSC-GRO™ Pancreatic Cancer-Associated Fibroblast (CAF) Maintenance Medium: 500mL
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Requirements for Establishing Cultures from Cryopreservation

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 N₂.

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, CO₂ cell culture incubator, calibrated to 5% CO₂. If the capabilities are available, we highly recommend growing the cells in hypoxic conditions (1 to 5% O₂). However, these cells can be cultured at ambient oxygen levels (~20% O₂) which results in reduced growth rates and passages. We highly recommend using Modular Incubation Chambers (MICs). Please contact technical services for information about various equipment and instrumentation options available to establish reduced oxygen cultures.



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Thawing 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.

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/cm² 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/Normal Fibroblasts. Data 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. 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% O₂, 5% CO₂, and with a N₂ balance at 37°C in a humidified environment. Monitor cell growth by visual inspection. When cultures become 80-90% confluent, proceed to subculture.

Subculturing

1. Once the cells are >80% confluent, wash the flask(s) 1-3 times with 1x PBS
2. After washing, add Accutase and incubate on orbital shaker with moderate agitation at 37°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.

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.



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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.
9. For routine passage, we recommend plating the cells at 5,000 to 10,000 cells/cm² 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.
- We recommend feeding every 3-5 days.
- 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!