NEUROMICS

Human Colorectal Tumor CAFs

Catalog #: CAF115

Cell #: >1x10^6 cells

Storage: Liquid Nitrogen until ready for culture.
While Culturing keep in 37°C CO_2 incubator

Product Format: Frozen Vial

GENERAL INFORMATION

Carcinoma associated fibroblasts (CAFs) have recently been implicated in important aspects of epithelial solid tumor biology such as neoplastic progression, tumor growth, angiogenesis, and metastasis.

Product is for Research use only.

Frozen Vials are shipped in a Dry Ice Package.

HANDLING OF ARRIVING CELLS

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

3. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

MEDIUM

We recommend customer use our Stem Cell Complete Low Serum Media (cat. CAFM01) to culture these cells. If you are looking for a faster growing culture, we recommend using our Stem Cell Complete Media (cat. CAFM02).

PROTOCOL FOR THAWING THE CELLS

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed. Decontaminate by dipping in or spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete low serum culture medium (cat. CAFM01) and centrifuge the cell suspension at approximately 125 x g for 5 to 10 minutes. Discard the supernatant.

4. Resuspend the cell pellet in the complete low serum culture medium at the dilution ratio recommended in the specific batch information Transfer to a 75 cm2 tissue culture flask. It is important to avoid excessive

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alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

SUBCULTURING PROCEDURE

Note: If you have any questions or need clarification regarding the protocol for culturing these cells, please reach out to Dr. Jensen Auguste at (978) 608-1766 with your questions before beginning.

Note: Volumes are given for a 75 cm2 flask. Increase or decrease the amount of medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1x PBS, remove and discard 1x PBS.
3. Add 2.0 to 3.0 mL of Cell Detachment solution (cat. ADF001) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
    - **Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. To remove cell detachment solution, transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh serum-free growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
6. Place culture vessels in incubators at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:6 is recommended

Medium Renewal: Every 2 to 3 days

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