NEUROMICS

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Vitro Biopharma
Harnessing the Power of Cells™

Human Proximal Tubule Epithelial – SC00A1-PTEC

Protocol for cell culture of human tubule epithelial cells derived mesenchymal stem cells manufactured by Vitro Biopharma

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. Storage in a –80°C freezer may be used but is likely to result in diminished cell viability proportional to storage time.

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 and 1% to 5% O_2. However, these cells can be cultured at ambient oxygen levels (~20% O_2) which results in reduced growth rates. Please contact technical services for information about various equipment and instrumentation options available to establish reduced oxygen cultures. Also, a water bath equilibrated to 37°C is needed. Required reagents include 1 x PBS, Fisher Catalog number BP665-1 or equivalent at room temperature or 37°C and MSC culture medium. We recommend using our MSC-GRO media (cat no. SC00B1) for optimal self-renwal and proliferation. This growth media is can be provide in low serum, humanized and serum free (cat no SC00B1, SC00B2 and SC00B3 respectively). Data that is presented on our website shows improved growth, 2-3-fold greater cellular recovery and substantially greater real time stability of our low serum MSC-Gro™ medium compared to similar products provided by Lonza, Stem Cell Technologies and InVitrogen. Other available MSC media may be used.

To establish cultures from frozen cell stock, it is first necessary to rapidly thaw cells at 37°C. Remove the desired number of vials containing cells from liquid nitrogen.

Exposure of closed vials containing liquid nitrogen to a 37°C water bath is an explosion hazard! It is essential to ensure that no liquid nitrogen is present in the vial! If liquid nitrogen is present in the vial, allow this to evaporate before proceeding. Please use proper precautions including appropriate gloves to protect skin from exposure to liquid nitrogen, eye protection and other personal protective equipment when transferring vials containing cryopreserved cells from liquid nitrogen to a 37°C water bath.

Provide continuous agitation, e.g., 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.**

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Count cells by a suitable method including a hemacytometer or automated cell counting device and determine the concentration of cells within the cell suspension. Our products are provided in 0.5 ml (500 l) of cryopreservation medium at a nominal concentration of 1 x 10^6 cells/ml. We recommend direct inoculation of cultures from the cell-cryopreservation media suspension. (Washout of the cryopreservative has been shown to decrease viability.)

We suggest establishing the culture at a plating density of about 7,500 to 10,000 cells/cm^2 in suitable tissue culture dishes or flasks. Add the appropriate volume of MSC culture medium to the plate or flask to be used for culture. We typically add 10 ml of medium per T-25. Use these guidelines to determine the appropriate volume of medium for your application.
Following inoculation with the appropriate volume of cell suspension, gently agitate the flask or plate to ensure homogeneous distribution of the MSCs with the cell culture medium. Allow cultures to incubate in 5% CO2 in ambient or reduced O2 as noted above at 37oC in a humidified environment. Monitor cell growth by visual inspection. When these cultures are 80% to 90% confluent, split and subculture the cells as described in the next section. This should require about 3 to 4 days of continuous culture, but this time depends on several factors. Thus the cultures should be monitored by inspection with an inverted microscope with appropriate magnification e.g., 100x

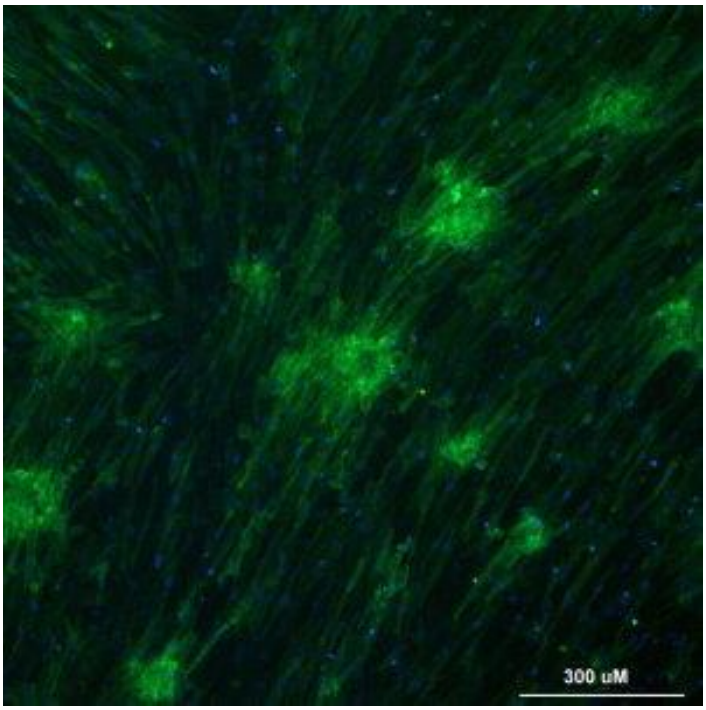

Image: Human Renal Proximal Tubule Epithelial Cells stained with CD-13 (green) and DAPI (Nuclei-blue).

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Subculture Procedures:
Wash each flask 2-3 times with PBS (e.g., 5 ml per T-25) and then add Accutase™ (Innovative Cell Technologies, Catalog number AT104 (4 mls/T-25 or 8 mls/T-75) and incubate at 37°C with gentle agitation for 15 minutes. (Alternatively, trypsin may be used instead of Accutase.) Visualize the culture. If necessary, assure complete detachment of cells as by rapping the flask or plate firmly on a solid surface. Transfer the dissociated cells to a centrifuge tube and combine this with a PBS wash of the flask (5ml/T-25; 10 ml/T75) followed by a second smaller volume wash. Centrifuge for 5-7 minutes at 450 x g and pour off or aspirate the supernatant. Resuspend the pelleted cells in 1 ml PBS by repetitive elutriation. Count the cells using an automated cell counter or hemacytometer. For automated counters, count in the size range 10 to 30 μm. Inoculate the cells at 4,000 to 5,000 cells/cm2 for routine passage. For optimal viability, complete the subculture process within 2 hours or less of dissociation. Fully adapted MSCs typically require about 4 to 5 days to reach about 90% confluence, although this is dependent on several different factors. We recommend feeding every three days. For longer or shorter periods between subculture, cultures may be inoculated at lower or higher densities. Subculture at lower or higher plating densities may also be used depending on the application. Our suggested procedures are provided as guidelines and may require adjustments within different laboratory environments.

Freeze-down Procedures:
Obtain an accurate count of the number of cells to be cryopreserved. Centrifuge these cells and aspirate the supernatant. Suspend the cells at the desired concentration, e.g., 1 million/ml, in cryopreservative medium. (10% DMSO is a suitable cryopreservative for these cells.) Transfer into appropriate cryopreservation vials that are rated for use in liquid nitrogen. Incubate the cells at room temperature for 30 minutes prior to freezing. Freeze the cell suspension at a slow rate, approximately 1oC/minute to ~ -80°C. After complete freezing, transfer vials of cells to liquid N2-containing Dewar flask preferably in the vapor phase for long-term storage at maximum viability

Note: Should any issues arise while using our cells, our team is here to help troubleshoot any issues. Our cells are backed by our one-time replacement or refund policy. Our recommended protocol including recommended products must be used to be eligible for replacement or refund. Cells that have been refrozen are no longer eligible for refund or replacement.

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