Human Alpha Motor Neurons – SC00A1-aMN

Protocol for cell culture of human neural lineages derived from 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 –80ºC freezer may be used but is likely to result in diminished cell viability proportional to storage time. NOTE: These cells can be cultured for 10 to 20 days and cryopreservation is not recommended. Do not thaw until ready for use.

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 37ºC, 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 37ºC is needed. Required reagents include 1 x PBS, Fisher Catalog number BP665-1 or equivalent at room temperature or 37ºC and Neural culture medium. We provide Alpha Motor Neuron maintaining media (PC00B7) for optimizing healthy cells and stability.

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

Exposure of closed vials containing liquid nitrogen to a 37ºC 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 37ºC water bath.

Provide continuous agitation, e.g., swirling, to the vial while it is submerged in the 37ºC 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.

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 50,000 to 75,000 cells/cm^2 in suitable tissue culture dishes or flasks. Please note that extracellular matrices are important in culture of all cells, especially attachment dependent cells. Differentiated neural lineages require laminin or fibronectin coated flasks. We recommend using Corning Biocoat Laminin Cellware (Catalog Number 354533). Add the appropriate volume of MSC culture medium to the plate or flask to be used for culture following the guidelines of the plate or flask manufacturer. We typically add 10 ml of medium per T-25. Use these guidelines to determine the appropriate volume of medium for your application.

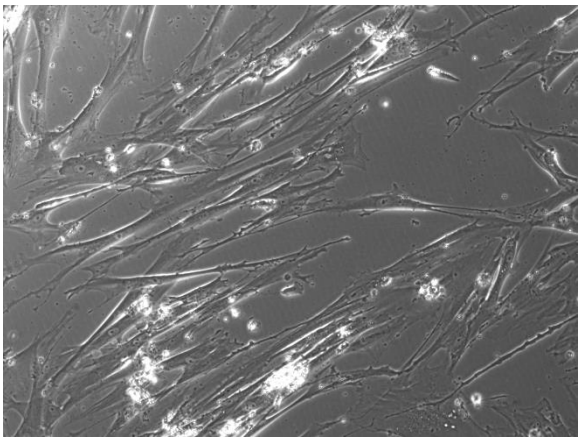
FOR RESEARCH USE ONLY

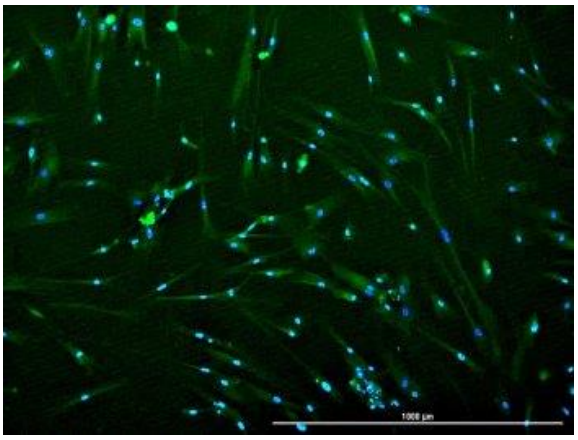
Neuromics Antibodies • 5325 West 74th Street, Suite 8 • Edina, MN 55439 phone 866-350-1500 • fax 612-677-3976 • e-mail pshuster@neuromics.com

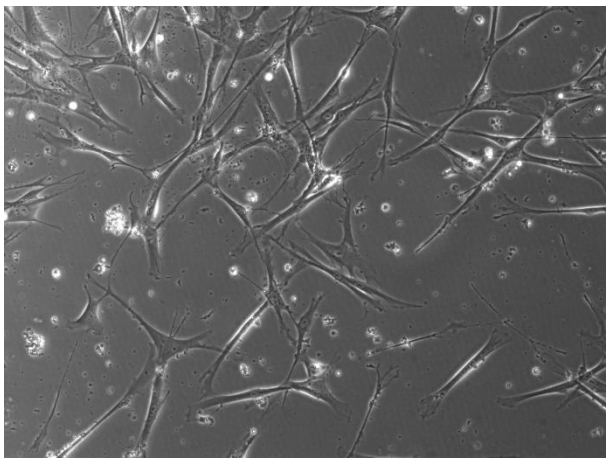
Following inoculation with the appropriate volume of cell suspension, gently agitate the flask or plate to ensure homogeneous distribution of the MSCs with the cell culture medium. Allow cultures to incubate in 5% CO_2 in ambient or reduced O_2 as noted above at 37ºC in a humidified environment. Monitor cell growth by visual inspection. This should require about 10 to 20 days of continuous culture, but this time depends on several factors (e.g. incubation conditions, media used, Rock inhibitor use, etc). Thus the cultures should be monitored by inspection with an inverted microscope with appropriate magnification e.g., 100x. Once cells become attached, within 3-5 days, change the media every other day until desired confluency is achieved. Testing and assay procedures can start after 1 week in culture with our maintaining media (Alpha Motor Neuron Maintaining media-PC00B7)

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.

Images: Human alpha Motor Neurons in Culture







FOR RESEARCH USE ONLY