Human Cerebellar Granule Cells (HCGC)

Catalog Number: 1530

Cell Specification

The development of the cerebellum involves a set of coordinated cell movements and two separate proliferation zones: the ventricular zone and the external granule cell layer (EGL), a rhombic-lip-derived progenitor pool [1]. The EGL appears to be segregated during early cerebellum formation and produces only granule cells. Cerebellar granule cells (CGC) are the most abundant neurons of the brain; about 101 billion in man [2]. Their axons run as parallel fibres along the coronal axis, and the one-dimensional spread of excitation that is expected to result from this arrangement is a key assumption in theories of cerebellar function. CGC receive inhibitory synaptic input from Golgi cells, which are mediated by gamma-aminobutyric acid (GABA). During both *in vivo* and *in vitro* development, CGC depend on the activity of the NMDA glutamate receptor subtype for survival and full differentiation [3]. Cultured CGC are widely used as a model system for studying neuronal apoptosis.

HCGC from ScienCell Research Laboratories are isolated from human cerebellum. HCGC are cryopreserved at primary culture and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. HCGC are characterized by immunofluorescent method with antibodies to neurafilament, MAP2, and beta-tubulin 3. HCGC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast and fungi. HCGC are guaranteed to further culture in the conditions provided by ScienCell Research Laboratories.

Recommended Medium

It is recommended to use neuronal medium (NM, Cat. No. 1521) for the culture of human cerebellar granule cells i*n vitro*.

Product Use

HCGC are for research use only. They are not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Transfer cells directly and immediately from dry ice to liquid nitrogen upon receiving and keep the cells in liquid nitrogen until cell culture is needed for experiments.

Shipping

Dry ice.

Reference

[1] Hatten, M. E. (1999) Central nervous system neuronal migration. *Annu. Rev. Neurosci.* 22, pp. 511–539.

[2] Andersen, B.B., Korbo, L. and Pakkenberg, B. (1992) A quantitative study of the human cerebellum with unbiased stereological techniques. J. Comp. Neurol., 326:549-560.

[3] Monti, B, Marri, L, Contestabile, A. (2002) NMDA receptor-dependent CREB activation in survival of cerebellar granule cells during in vivo and in vitro development. Eur J Neurosci. 16:1490-8.

Instruction for culturing cells

Caution: Cryopreserved cells are very delicate. Thaw the vial in a 37°C waterbath and return them to culture as quickly as possible with minimal handling!

Unpacking

1. For crypreserved cells: If there is dry ice in the package and you are not going to culture cells right way, place cryovial(s) immediately into liquid nitrogen. If there is no dry ice left in the package, thaw and culture the cells immediately.

2. For proliferating cells: Spray the culture vessel (flask, plate or slide) with 70% ethanol for disinfection. Transfer the cells into 37°C, 5% CO_2 incubator and allow equilibrating for 2 hours. After cells have equilibrated, remove shipping medium from the culture vessel and replace with fresh medium.

Set up culture after receiving the ordering

1. Coat culture vessel with laminin or poly-L-lysine.
   Note: It is important that neurons are plated in laminin or poly-L-lysine coated culture vessels that promote cell attachment and neurites outgrowth (poly-L-lysine coating: coat flask or plate with poly-L-lysine at 2 μg/ml concentration for one hour and wash the flask or plate with sterile water three times).

2. Medium preparation: Decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with the medium to recover the entire volume.

3. Set up culture: Prepare one T-75 flask for each cryovial. Add the appropriate amount of medium to the vessel (recommend for 20 ml/T-75 flask) and allow the flask to equilibrate in 37°C, 5% CO_2 incubator for at least 30 min.

4. Thawing of cells: Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field. Rinse the vial with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers.

5. Using 1 ml eppendorf pipette gently resuspend the cells in the vial and transfer them to equilibrated culture vessels (a T-75 flask). A high seeding density (>10,000/cm^2) is recommended.
   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange. Return the culture vessels to the incubator.

7. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A health culture will display normal neuron morphology, and nonvacuole cytoplasm with multiple processes.

Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, proper precautions mush be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. J Tissue Culture Methods. 11(4).