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 HCGC 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!

Set up culture after receiving the ordering:

1. Prepare a poly-L-lysine coated flask (2 μg/cm^2, T-45 flask is recommended). Add 5 ml of sterile water to a T-45 flask and then add 9 μl of poly-L-lysine stock solution (10 mg/ml, ScienCell cat. no. 0413). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: 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 medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 15 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. 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, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 20,000 cells/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. It is also important that HCGC are plated in poly-L-lysine coated culture vessels that promote cell attachment.
6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. 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.

Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells.

2. Change the medium every two to three days thereafter.

It is not recommended that HCGC be subcultured beyond their initial plating.

Caution: Handling human derived products is potentially bioharzadous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, 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).