Human Cerebellar Granule Cells (HCGC)

Catalog #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 segregated during early cerebellum formation and produces only granule cells. Cerebellar granule cells (CGC) are the most abundant neurons in the brain, about 1 x 10^{11} in humans [2]. Their axons run as parallel fibres along the coronal axis, and the one-dimensional spread of excitation that results 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 P0 and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. HCGC are characterized by immunofluorescence with antibodies specific to β-tubulin III. HCGC are negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi. HCGC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *HCGC are not recommended for expanding or long-term cultures since the cells do not proliferate in culture*.

Recommended Medium

It is recommended to use Neuronal Medium (NM, Cat. #1521) for culturing 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

Upon receiving, directly and immediately transfer the cells from dry ice to liquid nitrogen and keep the cells in liquid nitrogen until they are needed for experiments.

Shipping

Dry ice.

References

[1] Hatten ME. (1999) "Central nervous system neuronal migration." *Annu Rev Neurosci.* 22: 511-39.
[2] Andersen BB, Korbo L, Pakkenberg B. (1992) "A quantitative study of the human cerebellum with unbiased stereological techniques." *J Comp Neurol*. 326: 549-60.
[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.

Instructions for culturing cells

Caution: Cryopreserved primary cells are very delicate. Thaw the vial in a 37ºC water bath and return the cells to culture as quickly as possible with minimal handling! Do not centrifuge the cells after thawing as this can damage the cells.

Note: *Experiments should be well organized before thawing HCGC.* ***HCGC cannot be subcultured or passaged, as the cells do not proliferate.***

Initiating the culture:

Note: ScienCell primary cells must be cultured in a 37°, 5% CO2 incubator. Cells are only warranted if ScienCell media and reagents are used and the recommended protocols are followed.

1. Prepare a poly-$_L$-lysine-coated culture plate (2 µg/cm^2 is recommended). For example, add 2 ml of sterile water to one well of a 6-well plate and then add 20µl of poly-$_L$-lysine stock solution (1 mg/ml, Cat. #0403). Leave the plate in a 37ºC incubator overnight (or for a minimum of one hour).

   Note: It is important that these cells are plated in poly-$_L$-lysine-coated culture vessels to promote cell attachment.

2. Prepare complete Neuronal Medium (NM, Cat #1521). Decontaminate the external surfaces of medium bottle and medium supplement tubes with 70% ethanol and transfer them to a sterile field. Aseptically transfer supplement to the basal medium with a pipette. Rinse the supplement tube with medium to recover the entire volume.

   Note: We recommend pre-warming the complete medium to room temperature (Not 37°C), prior to use.

3. Prepare a 50 ml conical tube with complete medium. We recommend adding the appropriate volume of complete medium to the conical tube, as shown in Table 1.

Table 1
Recommended medium volume for cell suspension using a 6-well, 12-well, or 24-well format

Well format	Neuronal medium volume added to the conical tube
6-well	18 ml
12-well	24 ml
24-well	24 ml

4. Leave the tube in the sterile field and proceed to thaw the cryopreserved cells.

5. Place the frozen vial in a 37ºC water bath. Hold and rotate the vial gently until the contents completely thaw. Promptly remove the vial from the water bath, wipe it down with 70% ethanol, and transfer it to the sterile field. Carefully remove the cap without touching the interior threads.

6. Gently dispense the contents of the vial into the conical tube containing the complete medium.

   Note: <u>One vial of cells is sufficient to thaw and plate onto an entire multiwell plate</u> (6-well, 12-well, or 24-well plate). Centrifugation of cells after thawing is not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture.

7. **Gently invert** the tube **1-2 times** to obtain a homogenous cell suspension in the medium. Resuspending primary neurons by pipetting up and down is NOT recommended.
8. Gently aliquot the recommended cell suspension volumes onto 6-well, 12-well, or 24-well plates, as shown in Table 2.

Table 2
Recommended cell suspension volume per vial using a 6-well, 12-well, or 24-well format

Well format	Volume of media/well	# of wells/vial
6-well	3.0 ml	6 wells
12-well	2.0 ml	12 wells
24-well	1.0 ml	24 wells

9. Replace the lid of the culture plate and gently rock the plate to distribute the cells evenly.
10. Return the culture vessel to the incubator.
11. For best results, do not disturb the culture for at least 16 hours after the culture has been initiated. Refresh culture medium the next day to remove residual DMSO and unattached cells, then every other day thereafter.
12. Cells may take a few days to grow neurites in culture.

It is not recommended that neurons be subcultured beyond their initial plating as these cells do not proliferate.

Caution: Handling human-derived products is potentially biohazardous. Although each cell strain tests negative for HIV, HBV, and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working with 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 WE, Polt S. (1988) "Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues." *J Tissue Cult Methods.* 11: 191-9.