Mouse Cerebellar Granule Cells (MCGC)

Catalog #M1530-57

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 of the brain [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.

MCGC from ScienCell Research Laboratories are isolated from postnatal day 8 C57BL/6 mouse cerebellum. MCGC are cryopreserved as primary cultures and delivered frozen. Each vial contains >1 x 10^6 cells in 1 ml volume. MCGC are characterized by immunofluorescence with antibodies specific to neurofilament, MAP2, and β-tubulin III. MCGC are negative for mycoplasma, bacteria, yeast, and fungi. MCGC are guaranteed to further culture under the conditions provided by ScienCell Research Laboratories; however, *MCGC 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 MCGC i*n vitro*.

Product Use

MCGC 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, pp. 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 cells are very delicate. Thaw the vial in a 37°C water bath and return the cells to culture as quickly as possible with minimal handling!

Initiating the culture:

1. Prepare a poly-L-lysine-coated culture vessel (2 µg/cm^2). It is recommended to use a T-25 flask (alternatively, 3 wells of a 6-well plate or 12 wells of a 24-well plate can be used). Add 5 ml of sterile water to a T-25 flask and then add 5 µl of poly-L-lysine stock solution (10 mg/ml, Cat. #0413). Leave the vessel in a 37°C incubator overnight (or for a minimum of one hour). Rinse the poly-L-lysine-coated vessel twice with sterile water prior to use.

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

2. Prepare complete medium. 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.

3. Add complete medium to the culture vessel. Leave the vessel in the sterile field and proceed to thaw the cryopreserved cells.

4. Place the frozen vial in a 37°C 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.

5. Gently resuspend and dispense the contents of the vial into the poly-L-lysine-coated culture vessel. A seeding density of 10,000-50,000 cells/cm^2 is recommended, with an optimal range of 20,000-25,000 cells/cm^2.

   Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of residual DMSO in the culture.

6. Replace the cap or lid of the culture vessel and gently rock the vessel to distribute the cells evenly. Loosen cap, if necessary, to allow gas exchange.

7. Return the culture vessel to the incubator.

8. 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.

Maintaining the culture:

1. Refresh supplemented culture 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 neurons be subcultured beyond their initial plating.

Caution: Handling animal-derived products is potentially biohazardous. 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.