Rat Cortical Neurons

Cat. No. : CP-R105

General Information

Species	Rat/SD rats
Tissue Type	Nervous system
Tissue	Cerebral cortex tissue
Cell Type	Neuron
Morphology	Neuronal-like
Growth Properties	Adherent
Storage Conditions	For long-term cryopreservation, cryovials should be stored in liquid nitrogen at −150°C to −196°C. Storage at −80°C is restricted to short-term interim use only.

Culture Conditions and Handling

Complete Medium	Rat Cortical Neuron Complete Medium[CM-R105]
Population Doublings	Not recommended for expansion or long-term culture
Subcultivation Ratio	Non-subculturable
Dissociation Reagent	0.125% trypsin
Coating Conditions	PLL (0.1 mg/mL)
Medium Renewal	Every 2 to 3 days
Freezing Medium	General Freezing Medium[PB180436]
Incubation Atmosphere	Air, 95%; CO_2, 5%
Temperature	37°C
Instructions	1. Check all containers for leakage or breakage. 2. Remove the frozen cells from the dry ice packaging and immediately transfer them to liquid nitrogen (liquid or vapor phase) for long-term cryopreservation. 3. Neuronal cells adhere poorly; culture vessels must be coated. These cells easily shrink and detach upon cold exposure. All reagents must be pre-warmed to 37°C, and observation time at room temperature should be minimized.
Subculturing Procedure	Neuronal Cell Dissociation

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Subculturing Procedure

1. Transfer all culture medium from the flask to a sterile centrifuge tube. Add pre-warmed (37°C) phosphate-buffered saline (PBS) to wash the cells 1–2 times.
2. Add 1 mL of 0.125% trypsin solution (prepared by diluting 0.25% trypsin 1:1 with PBS, equilibrated to room temperature) to the flask. Gently tilt the flask to coat the entire bottom surface with trypsin, then incubate at room temperature for 4–5 min, gently swirling the flask every 2 min. After digestion, gently tap the flask and examine cell detachment under an inverted microscope (stop digestion once neuronal cells begin to detach).
3. Once most cells have detached, add 4 mL of medium supplemented with 10% FBS to neutralize digestion. Do not pipette against the flask bottom to dislodge residual cells; only collect the cell suspension into a centrifuge tube. If a substantial number of cells remain adherent, rinse the flask with PBS, collect the rinse into the centrifuge tube, and repeat digestion 1–2 times. Collect and pool cells from each digestion round.
4. Centrifuge at 1200 rpm for 5 min and discard the supernatant. Add 5 mL of PBS to gently resuspend the cells, then centrifuge again at 1200 rpm for 5 min and discard the supernatant. Add an appropriate volume of complete neuronal medium to resuspend the cells, and seed into the required culture vessels (culture vessels must be pre-coated with poly-L-lysine). Recommended seeding density: 1–1.2 × 10^5 cells/cm² (adjust according to experimental requirements).
5. Incubate statically for 24–48 h until cells adhere and fully spread out; the cells are then ready for downstream experiments.

Note: Each digestion round must not exceed 5 min. Collect detached cells every 5 min, and repeat the digestion procedure for remaining cells as described above.

Background

Rat cortical neurons (*Rattus norvegicus*) are isolated from cerebral cortex tissue. They are the most predominant excitatory neurons in the central nervous system. These cells exhibit a multipolar morphology and possess typical dendritic and axonal structures. They express microtubule-associated protein 2 (MAP2). Their core functions include: generating and conducting nerve impulses; forming synaptic connections to participate in information processing and transmission; and regulating higher nervous activities of the cerebral cortex. Injury and apoptosis of rat cortical neurons are closely associated with stroke and Alzheimer's disease. In vitro-cultured rat cortical neurons serve as an important model for investigating neural development and neurodegenerative diseases.

Rat cortical neurons isolated from Procell Laboratory are prepared by trypsin digestion, followed by selective culture in specialized neuronal medium and chemical reagent inhibition. Each vial contains approximately 5 × 10^5 cells.

Rat cortical neurons isolated from Procell Laboratory exhibit positive immunofluorescence staining for β-Tubulin-III, with a purity greater than 90%. In addition, the cells test negative for HIV-1, HBV, HCV, mycoplasma, bacteria, yeast, and fungi.

Handling Recommendations for Cryopreserved Cells

Preparations Before Thawing

1. Primary cells have high nutritional demands; therefore, prepare complete culture medium in advance.
2. Preheat the complete culture medium to 37°C for 30 minutes.

3. Prepare 9 mL of complete medium within a sterile centrifuge tube to dilute the cryoprotectant present in the frozen cell suspension.
4. Use sterile gloves to protect the cryovial from contact with the water bath water, and preventing cell contamination.

Thawing Procedure

1. Retrieve the cryovial containing frozen cells from liquid nitrogen storage. Place it in sterile gloves and immediately submerge it in a 37°C water bath.
2. Thaw the cells rapidly (<1 minute) by gently swirling the vial in the 37°C water bath until only a small ice crystal remains.
3. Transfer the vial to a laminar flow hood. Wipe the exterior of the vial with 75% ethanol before opening.
4. Transfer the thawed cells dropwise into a preheated 9 mL centrifuge tube containing complete medium.
5. Centrifuge the cell suspension at approximately 250 × g for 5 minutes. For cells characterized by low density and small volume, such as lymphocytes and suspended cells, the centrifugation speed can be appropriately increased to 400 × g for 8 minutes.
6. After centrifugation, discard the supernatant and retain the cell pellet.
7. Gently resuspend the cells in complete growth medium, then transfer them to an appropriate culture vessel and place it in the recommended culture environment.

Notes

1. The entire recovery process should be completed as quickly as possible.
2. Select the culture vessel size based on the number of cells in the cryovial. Procell single cryovials are recommended for resuspension in 6 cm dishes or T25 flasks.
3. Minimize the time thawed cells are kept at room temperature. DMSO must be immediately diluted or removed by centrifugation.

Precautions After Receipt of Frozen Cells

1. Upon unpacking, inspect the condition of the frozen cells and dry ice, and take photographs immediately. The following after-sales service will be provided based on these photographs, including assessment of the remaining dry ice, verification that the cryovial was fully buried in dry ice, and evaluation of whether the cells thawed and refrozen during transit.
2. Upon receipt, the cells should be transferred to liquid nitrogen immediately or directly resuscitated. If liquid nitrogen is unavailable, the cells can be temporarily stored at -80°C, however, the storage period should be limited to less than one week whenever possible. Prolonged storage at -80°C may gradually reduce post-thaw cell viability, and the extent of this viability loss is unpredictable.
3. Ensure that the operator has sufficient knowledge and experience in cell culture, and the laboratory is equipped with essential instruments, including a biosafety cabinet, CO_2 incubator, inverted microscope, centrifuge, water bath. Carefully review the cell instruction manual to understand key cell characteristics including growth properties (adherent or suspension), morphology, basal medium requirements, serum concentration, cytokine supplementation, subcultivation ratio, medium renewal schedule.
4. After resuscitation, observe the cells under a microscope and record the cell status by taking photographs (1-3 images each at 100× and 200× magnification for 3 consecutive days). These images will serve as supporting documentation for follow-up services. In addition, a small aliquot of cells may be used to assess cell viability by automated cell counting or trypan blue staining.

Note: Cells should not be observed too frequently within the first 24 hours after resuscitation, as this may affect cell growth or adherence. Observation once per day is sufficient.

5. Primary cells have a limited number of passages; it is recommended that they be used as soon as possible.

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