NEUROMICS

Frozen Primary Mouse Neurons Datasheet and Protocol

Catalog Number: PC37000 **Product Type:** Cryo-preserved Neurons

Source: E18-E19 C57/BL6 Mouse Brain

Format: Cryo-preserved vials.

Storage: -80^{0}C for short term storage. Cryo-preserve for long term storage.

Protocol

Introduction

When growing neurons in serum-containing medium, they must be in contact with astrocytes if they are to survive for long periods of time in culture. However, the glial proliferation can complicate the longer-term culture of neurons. In addition, neurons can lose proliferation potential when grown in serum-containing medium and will enter a growth crisis phase. If the cultures are continued in serum-containing medium, genetic abnormalities can occur such as tetraploidy or tumorigenic potential. Therefore it is recommended to culture neurons in serum-free medium that is supplemented with special nutrients.

Primary Mouse Neural Culture Medium (Catalog # M37105) is a serum-free DMEM/F12-based medium containing L-glutamine, HEPES, Penicillin, Streptomycin and proprietary factors that increases neuron viability. Primary Culture Media Supplement (Catalog # M37106) is a nutrient supplement to be used in conjunction with Primary Neural Culture Media (Human, Rat and Mouse). It contains insulin, transferrin, human high-density lipoprotein (HDL), selenium plus additional factors important in maintaining neuronal health. It is supplied as a sterile 100X solution.

It is not advisable to dilute media supplements such as insulin, transferrin, human high-density lipoprotein (HDL) and selenium into culture medium stocks as their stabilities decrease and they tend to bind to polystyrene plastic. Instead, add the supplements directly to tissue culture vessels upon passaging. In addition, it is recommended to add cytosine β-D-arabinofuranoside (Ara-C) (Sigma, Catalog # C1768) after 4-5 days of plating to avoid astrocyte proliferation. If astrocytes are desired, this step can be omitted.

Coating Plates

Neurons are plated onto culture vessels that been pre-coated with either poly-L-lysine and fibronectin or poly-L-lysine and laminin. Coating is best achieved with poly-L-lysine that has a molecular weight between 30-70 kDa.

Coating Steps

1. Dissolve 10 mg poly-L-lysine hydrobromide in 1 ml sterile, de-ionized water. This creates a 1% stock solution that can be stored at 4°C for a few days or at -20°C for a few months. Only prepare the amount of 1X coating solution from the stock solution intended for use at one time.

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2. Dilute the poly-L-lysine stock solution in PBS to a final concentration between 0.3 to 0.5%.

3. Coat substrates with enough final poly-L-lysine coating solution to completely cover the surface, typically 1-2 ml/25 cm^2.

4. Rock gently to ensure even coating of the surface. Leave for 10-15 minutes at room temperature.

5. Remove the poly-L-lysine coating solution and immediately rinse culture vessels twice with PBS. At this point, poly-L-lysine-coated vessels may be stored at 4°C for several months before coating with fibronectin or laminin.

6. After coating with poly-L-lysine, add fibronectin or laminin to culture vessels at 10 ug/ml in serum-free Primary Mouse Neural Culture Medium (Catalog # M37105) without Primary Culture Media Supplement (Catalog # M37106) for 1-2 hours at 37°C in a 5% CO_2 atmosphere. Add just enough fibronectin or laminin solution to cover the bottom of the dish or flask.

7. Following pre-coating, aspirate the fibronectin or laminin solution and wash once with fresh serum-free medium without nutrient supplements. Pre-coated vessels can be stored at this point for several days at 4°C.

Plating and Passaging Cells

When passaging neurons, it is necessary to remove the cells from the tissue culture plastic. This can be accomplished by using a standard trypsin solution or by using a non-enzymatic dissociation solution. A 0.2-0.3% trypsin-EDTA solution is used followed by a trypsin inhibition step. Trypsinization is a faster method, but can damage neurons if they are left too long in trypsin solution; therefore, non-enzymatic dissociation solutions are preferred for maintaining neuron viability. Neuromics provides a Non-enzymatic Cell Dissociation Solution (Catalog # 37108) that can be used instead of trypsin. It is supplied as a 1X ready-to-use sterile solution.

Passaging Steps

1. Pre-warm the non-enzymatic cell dissociation solution to 37°C.

2. Aspirate the culture medium from the culture vessel. Slowly add a small volume of non-enzymatic cell dissociation solution to the culture vessel (e.g. 2 ml/cm^2). Gently swirl the vessel to cover the cells.

3. Incubate the cells in dissociation solution for several minutes and monitor the dissociation. The amount of time to dissociate the neurons varies and can range from 5-20 minutes.

4. Place the solution containing the cells in a centrifuge tube and centrifuge for 5 minutes at 500g (at room temperature or at 4°C)

5. Remove the supernatant and re-suspend the cells in a small volume of Primary Mouse Neural Culture Medium (Catalog # M37105). Perform a cell count.

6. Prepare a cell suspension of desired density of viable cells by diluting the cell suspension in serum-free Primary Mouse Neural Culture Medium (Catalog # M37105).

7. Plate at a density between $1–5 \times 10^5$ cells/cm^2 depending on the application. Plating at a lower density is recommended in general as it requires less frequent feeding and the cultures tend to be healthier. The media in neuronal cultures will often become hazy or murky especially at higher densities, which is normal.

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8. Add 6 to 8 µM Ara-C to inhibit non-neuronal growth if desired. Upon thawing cells and starting cultures, do not add Ara-C until 3-4 days after cultures are established.

9. Four to seven days after plating (depending on the plating density), place serum-free Primary Mouse Neural Culture Medium (Catalog # M37105) in the incubator for 30 min to allow it to equilibrate to 37°C and 5% CO_2.

10. Carefully aspirate off the used media and replace with pre-warmed fresh medium.

11. After the initial media change, replace 50% of the growth media every 3-5 days depending on the density.

12. Continue to incubate plates for up to 3 to 4 weeks, replacing 50% of the growth media every 3-5 days depending on the density.

Cryopreservation of Neurons

Neurons can be cryopreserved for long periods of time if suspended properly in cryopreservation medium and stored in liquid nitrogen. The Primary Neural Culture Cryopreservation Media (Catalog # M37107) is a derivative of Primary Mouse Neural Culture Medium that contains 15% DMSO.

Cryopreservation Steps

1. Pre-warm the non-enzymatic cell dissociation solution to 37°C.

2. Aspirate the culture medium from the culture vessel. Slowly add a small volume of non-enzymatic cell dissociation solution to the culture vessel (e.g., 1-2 ml per T-25 flask). Gently swirl the vessel to cover the cells.

3. Incubate the cells in dissociation solution for several minutes and monitor the dissociation. The amount of time to dissociate the neurons varies, but can range from 10-20 minutes.

4. Place the solution containing the cells in a centrifuge tube and centrifuge for 5 minutes at 500g (at room temperature or at 4°C)

5. Resuspend the cells in Primary Neural Culture Cryopreservation Media (Catalog # M37107) at a density of 10^6-10^7 cells/ml and transfer to cryogenic vials.

6. Place the vials at -80°C in a Styrofoam container, which allows a gradual freezing process. Leave the vials at -80°C overnight.

7. The following day, quickly transfer the cryogenic vials to liquid nitrogen.

Reinitializing Cultures

Reinitializing Steps

1. Remove a vial of cells from liquid nitrogen and thaw quickly in a 37°C water bath.

2. Place the cryoprotective solution containing the cells in a centrifuge tube and centrifuge for 5 minutes at 200g (at room temperature or at 4°C)

3. Remove the supernatant and re-suspend the cells in a small volume of Primary Mouse Neural Culture Medium (Catalog # M37105). Perform a cell count.

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4. Prepare a cell suspension of desired density of viable cells by diluting the cell suspension in serum-free Primary Mouse Neural Culture Medium (Catalog # M37105). Plate at a density between 1–5 × 10^5 cells/cm^2 depending on the application. Plating at a lower density is recommended in general as it requires less frequent feeding and the cultures tend to be healthier.

5. Continue culturing the cells as described above.

Reagents and Media Needed Not Provided

- Poly-D-lysine hydrobromide
- Fibronectin or Laminin
- Trypan blue to count viable cells
- Sterile pipette tips
- Sterile centrifuge tubes
- General cell culture supplies (culture plates, cryogenic vials, etc.)
- Primary Mouse Neural Culture Medium (Catalog # M37105)
- Primary Culture Media Supplement (Catalog # M37106)
- Primary Neural Culture Cryopreservation Media (Catalog # M37107)
- Non-enzymatic Cell Dissociation Solution (Catalog #M37108)

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