

Rat Dorsal Root Ganglion Neurons

Instructions for Use

Receiving Instructions: Unpack immediately! Packages may contain components with various storage requirements!

Safety

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use *in vitro* diagnostic or clinical procedures. **WARNING:** Handle as a potentially biohazardous material under Biosafety Level 1 containment. These cells are not known to contain an agent known to cause disease in healthy adult humans. These cells have not been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. If you require further information, please contact your site Safety Officer or Scientific Support.

Unpacking and Storage Instructions

1. Cells should be stored in liquid nitrogen. Do NOT store cells at -80°C. The cells are extremely temperature-sensitive and should be transferred to liquid nitrogen **immediately** upon arrival. Cells should be transported on dry ice or in a liquid nitrogen container. When transporting the cells on dry ice make sure that the vials are **completely** covered.
2. Upon arrival, store Basal Medium at 4°C protected from the light. Store SingleQuots™ at -20°C in a freezer that is not self-defrosting. Once the medium is supplemented with L-Glutamine and GA it may be stored for up to 4 weeks at 4°C.

Preparation of Medium

The recommended medium for Rat Dorsal Root Ganglion Neurons is the PNGM™ BulletKit™. The BulletKit™ contains a 200 ml bottle of Primary Neuron Basal Medium (PNBM) and PNGM™ SingleQuots™. The fully supplemented media contains 2mM L-Glutamine, 50 ug/ml Gentamicin/37 ng/ml Amphotericin, and 2% NSF-1. It is **strongly recommended** that NSF-1 be aliquoted, frozen and then added to the media as needed immediately before each use.

1. Thaw the SingleQuots™ at room temperature.
2. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
3. Aseptically open the L-Glutamine and GA vials and add the entire amount to the basal medium with a pipette.
4. Rinse the empty vials with medium. It may not be possible to recover the entire volume listed, but small losses will not affect the cell growth characteristics of the medium.
5. Transfer the desired volume of medium to a sterile secondary container and add NSF-1 for a final concentration of 2%. **For Example:** Add 1.0 ml of NSF-1 to 49 ml of media.

Note: If there is a concern that sterility was compromised during this process, the medium may be filtered with a 0.2 µm filter to assure sterility. Routine refiltration is not recommended. Filtration after the addition of NSF-1 is not recommended.

6. Aliquot remaining NSF-1 at desired volume (e.g. 3 x 1 ml) and store at -20°C.
7. Thaw individual NSF-1 aliquots as needed to prepare fresh media. Additional freeze-thaw cycles are not recommended.

Note: To promote optimal survival of embryonic neurons, the osmolality of PNBM is lower (210-240 mOsm/kg H₂O) than many classic cell culture media. The specific osmolality of each lot of PNBM is noted on the Certificate of Analysis. Supplementation of PNBM with PNGM™ SingleQuots™ typically increases the osmolality by approximately 10 mOsm/kg H₂O. To avoid osmotic shock, the osmolality should be taken into account if cells are transferred to other media or salt solutions.

Coating Plates

Primary neuronal cells need an appropriate substrate to adhere and survive. The preferred substrate is poly-D-lysine with laminin. Poly-D-lysine can also be used alone to coat the cell culture plasticware or cover slips. Coated cell culture plates, dishes, or cover slips can either be purchased from a supplier or prepared immediately prior to use. Protocols for the recommended substrates are available on our web site at www.lonza.com.

Thawing of Cells / Initiation of Culture Process

Note: Doing a trypan blue viability count upon thaw is not recommended as live cells will also uptake the dye.

1. DAY 1: Remove a vial of cells from liquid nitrogen and place in a water bath pre-heated to 37°C. **IMPORTANT:** Do not centrifuge or vortex the cells. Keep the time between removing the vial from the liquid nitrogen tank and placing into a pre-heated water bath as short as possible.
2. After 1½ minutes, remove vial and disinfect the outside of the vial by wiping with 70% ethanol. Place in a laminar flow hood. Proceed with the next step immediately after thawing.
3. Gently transfer 0.25 ml cells into a 15 ml centrifuge tube and immediately add pre-warmed medium (volume of 7.75 ml, as indicated in table below) drop-wise onto the cells, while rotating the tube by hand. This should take approximately 2 minutes. **IMPORTANT:** Do not add the whole volume of medium at once to the cells. This may result in osmotic shock. If one vial of cells is to be used for several different experiments at one time, mix the cells first by pipetting slowly up and down once, then aliquot the cells into the appropriate vessels.
4. Mix cell suspension by inverting the tube carefully, twice. **IMPORTANT:** Do not vortex the cells.
5. Transfer cell suspension to appropriate flasks, petri dishes or well plates. See chart below for recommended volumes of medium.
6. Incubate the cells for 4 hours in a 37°C, 5% CO₂ incubator.
7. Remove the medium from the cells leaving a small volume to ensure the cells do not dry out and add fresh, pre-warmed medium.
8. For inhibition of Schwann cell proliferation adds mitotic inhibitors (see Preparation of

Mitotic Inhibitors) to the wells at the 4-hour period and every time a media change is performed. Add 5 µl of each inhibitor per ml of media. (17.5 µg/ml uridine and 7.5 µg/ml of 5-fluoro-2'-deoxyuridine) **NOTE:** In the absence of mitotic inhibitors during the first 7 days *in vitro*, the neurons will cluster (ganglionate) and detach from the substrate.

9. Incubate the cells at 37°C with 5% CO₂.

Cell death will be observed; Cultivation of the cells should be continued.

10. DAY 5: Change the medium.
11. For a longer period of cultivation, replace 50% of the media with fresh, pre-warmed media containing mitotic inhibitors every 3 to 4 days.

Volume of Medium	Plating Format
7.75 ml	0.25 ml cells suspension
200 µl/well	96-well plate
1 ml/well	24-well plate

Preparation of Mitotic Inhibitors

The following procedures should be performed in a Biological Safety Cabinet.

5-fluoro-2'-deoxyuridine

1. Weigh 15 mg of 5-fluoro-2'-deoxyuridine (Sigma cat # F0503) and place into a 15 ml conical tube.
2. Add 10 ml sterile distilled deionized water to the tube and mix well.
3. Remove the plunger from a 20 cc sterile syringe. Attach a sterile 0.2 µm syringe filter onto the syringe. Place the tip of the filter into a new sterile 15 ml centrifuge tube. Pour the 5-fluoro-2'-deoxyuridine solution into the syringe and replace the plunger.
4. Press the plunger down to push the 5-fluoro-2'-deoxyuridine solution through the filter.
5. Using a 10 ml sterile pipette, aliquot the sterile 5-fluoro-2'-deoxyuridine in 1 ml aliquots into sterile Eppendorf™ Tubes. Place in a -20°C freezer until needed.

Uridine

1. Weigh 35 mg of 1-β-D-ribofuranosyluracil (Sigma Cat. No. U-3003) into a 15 ml centrifuge tube.
2. Add 10 ml sterile distilled deionized water to the tube and mix well.

3. Remove the plunger from a 20 cc sterile syringe. Attach a sterile 0.2 μm syringe filter onto the syringe. Place the tip of the filter into a new sterile 15 ml centrifuge tube. Pour the uridine solution into the syringe and replace the plunger. Press the plunger down to push the uridine solution through the filter.
4. Using a 10 ml sterile pipette, aliquot the sterile uridine in 1 ml aliquots into sterile Eppendorf™ Tubes. Place in a -20°C freezer until needed.

Maintenance

1. After initial media change on day 5, replace 50% of the growth media every 3 to 4 days.
2. Warm an appropriate amount of medium to 37°C in a sterile container. Remove 50% of the medium from the cell culture. Replace with the warmed, fresh medium and return the cells to the incubator.
3. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer only the required volume to a sterile secondary container.
4. Compensation for medium loss due to evaporation should be taken into consideration. Add additional medium whenever necessary.

Ordering Information

R-DRG-505	Rat Dorsal Root Ganglion Neurons (DRG)	≥ 0.25 ml cell suspension
CC-4461	PNGM™ BulletKit™	Kit which contains a 200 ml bottle of PNBM and PNGM™ SingleQuotes™
CC-3256	PNBM Basal Medium	Primary Neuron Basal Medium (200 ml)
CC-4462	PNGM™ SingleQuotes™	NSF-1, 4 ml; L-Glutamine, 2 ml; GA, 0.2 ml

Product Warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza guarantees cell performance only when the approved media and supplements are used.

Quality Control

The cells test negative for mycoplasma and bacteria. Additional molecular and immunochemical testing for quality is done following conditions that mimic shipping. When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website or our current catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.