

## Rat Brain Cerebellar Neurons

### Instructions for Use

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**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

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.

### Preparation of Medium

The recommended medium for the Rat Brain Cerebellar Neurons is the PNGM™-A BulletKit™. The BulletKit™ contains a 200 ml bottle of Primary Neuron Basal Medium (PNBM), PNGM™ SingleQuotes™, and PNGM™-A SingleQuotes™. 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 SingleQuotes™ 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, GA, OA and PA 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.

### Coating Plates

Primary neuronal cells need an appropriate substrate to adhere and survive. The preferred substrate is poly-D-lysine. 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](http://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 2½ 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.

- Gently transfer 1 ml cells into a 15 ml centrifuge tube and immediately add pre-warmed medium (volume of 7 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.
- Mix cell suspension by inverting the tube carefully, twice. **IMPORTANT:** Do not vortex the cells.
- Transfer cell suspension to appropriate well plate. See chart below for recommended volumes of medium.
- Incubate the cells for 4 hours in a 37°C, 5% CO<sub>2</sub> incubator.
- Remove the medium from the cells leaving a small volume to ensure the cells do not dry out and add fresh, pre-warmed medium.
- Incubate the cells at 37°C with 5% CO<sub>2</sub>.

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

- DAY 4: Change 50% of the medium. For inhibition of non-neuronal cell proliferation, add 2 µl of Cytosine Arabino Fluronoside per 1 ml of media.
- For a longer period of cultivation, replace 50% of the media with fresh, pre-warmed media containing 1ul mitotic inhibitors per 1 ml every 3 to 4 days.

| Volume of Medium | Plating Format        |
|------------------|-----------------------|
| 7 ml             | 1 ml cells suspension |
| 200 µl/well      | 96-well plate         |
| 1 ml/well        | 24-well plate         |

**Recommended Mitotic Inhibitors**

Cytosine Arabino Fluronoside from Sigma. Product number C-1768, 4 mM stock (1 mg/ml)

**Maintenance**

- After initial medium change on day 4, replace 50% of the growth medium every 3 to 4 days.
- 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.

- 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.
- Compensation for media loss due to evaporation should be taken into consideration. Add additional media whenever necessary.

**Ordering Information**

|                 |                              |   |
|-----------------|------------------------------|---|
| <b>R-CB-503</b> | Rat Brain Cerebellum Neurons | ≥ 1.0 ml cell suspension  |
| <b>CC-4512</b>  | PNGM™-A BulletKit™           | Kit which contains a 200 ml bottle of PNBM, PNGM™ SingleQuots™ and PNGM™-A SingleQuots™ |
| <b>CC-3256</b>  | PNBM Basal Medium            | Primary Neuron Basal Medium (200 ml)  |
| <b>CC-4462</b>  | PNGM™ SingleQuots™           | NSF-1, 4 ml; L-Glutamine, 2 ml; GA, 0.2 ml  |
| <b>CC-4511</b>  | PNGM™-A SingleQuots™         | OA, 0.5ml; PA, 1.5 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.