

Clonetics® Rat Brain Hypothalamic Neuronal Cells

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

1. Thaw the SingleQuots® at 4°C.
2. Decontaminate the external surfaces of all supplement vials and the media 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 media 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.

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 medium. 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 hypothalamic neuronal cells need an appropriate substrate to adhere and survive. The recommended substrate is poly-D-lysine with laminin. Coated cell culture plates, dishes, or cover slips can either be purchased from a supplier or prepared immediately prior to use. The protocol for the recommended substrate is available on our web site at www.lonza.com.

Thawing of Cells / Initiation of Culture Process

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 the pre-heated water bath as short as possible.
2. After 1 minute, remove the vial and disinfect the outside by wiping with 70% ethanol. Work in a laminar flow hood. Proceed with the next step immediately after thawing.
3. Gently transfer 0.5 ml cells into a 15 ml centrifuge tube and immediately add pre-warmed medium 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.

Note: We do not recommend performing a trypan blue viability count on the cells since some live cells will also uptake the dye. Cells should be thawed and plated directly.
6. Incubate the cells for 4 hours at 37°C in a 5% CO₂ incubator.
7. Remove the medium from the cells leaving a small volume to ensure the cells do not dry out. Add fresh, pre-warmed medium.
8. Incubate the cells at 37°C with 5% CO₂.

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

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

Volume of Medium	Plating Format
7.5 ml	0.5 ml cell suspension
200 µl/well	96-well plate
1 ml/well	24-well plate

Maintenance

1. After initial medium 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-HTH-507	Rat Brain Hypothalamic Neurons	≥ 0.5 ml cell suspension
CC-4461	PNGM™ BulletKit®	Kit which contains a 200 ml bottle of PNBM and PNGM™ SingleQuots®
CC-3256	PNBM Basal Medium	Primary Neuron Basal Medium (200 ml)
CC-4462	PNGM™ SingleQuots®	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.