



## Clonetics™ Rat Retinal Cell System

### R-Ret – Instructions for Use

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#### Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. If no dry ice remains, please contact Customer Service. Do NOT store cells at -80°C. The cells are extremely temperature-sensitive and should be transferred to liquid nitrogen or be used 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 the vials are completely covered.
3. BulletKit™ Medium instructions: store basal medium at 2°-8°C and SingleQuotes™ Kit at ≤20°C in a freezer that is not self-defrosting. Once thawed, SingleQuotes™ Kit should be stored at 2°-8°C and added to basal medium within 72 hours. After SingleQuotes™ Kit is added to basal medium, use within 1 month. Do not re-freeze. Using medium or reagents other than what is recommended will void the cell warranty. Please contact Scientific Support if you need help selecting media and/or reagents.

#### Preparation of Media

The recommended medium for the rat retinal cells is the PNGM™ BulletKit™. The BulletKit™ contains a 200 ml bottle of primary neuron basal medium (PNBM) and PNGM™ SingleQuotes™. It is strongly recommended that NSF-1 be aliquoted, frozen and then added to the media as needed immediately

before each use. The fully supplemented media contains 2mM L-glutamine, 0.2 ml GA-1000, and 2% NSF-1. It is recommended to use this medium containing 5% heat inactivated FBS for initial plating and short term culture (up to four days) and change to serum free medium for long term culture (≥5 days).

1. Thaw the SingleQuotes™ and FBS at room temperature or overnight in a 2-8°C refrigerator.
2. Decontaminate external surfaces of all vials and the medium bottle with ethanol or isopropanol.
3. To formulate Primary Neuron Growth Media (PNGM™ Medium) transfer the entire contents of the L-glutamine and GA-1000 vials of the PNGM™ SingleQuotes™ Kit (Catalog No. CC-4462) to the PNBM™ Basal Medium with a pipette, and rinse each vial with medium.
4. When preparing these BulletKit™ Media, it may not be possible to recover the entire volume listed for each vial. Small losses (up to 10%) should not affect the cell growth characteristics of the supplemented medium.
5. Transfer the label provided with each kit to the basal medium bottle(s) being supplemented (avoid covering the basal medium lot # and expiration date). Use it to record the date and amount of each supplement added.
6. PNGM™ Medium must be further supplemented NSF-1. Transfer the desired volume of medium to a sterile secondary container and add NSF-1 from the PNGM™ SingleQuotes™ Kit (Catalog No. CC-4462) for a final concentration of 2%. For Example: Add 80.00 µl of NSF-1 to 3.92 ml of media.

7. Transfer the desired volumes of complete PNGM™ Medium containing NSF-1 to a sterile container and add heat inactivated FBS to a final concentration of 5%.

**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.**

8. Aliquot remaining NSF-1 and FBS at desired volumes and store at -20°C
9. Thaw individual NSF-1 and FBS aliquots as needed to prepare fresh media. Additional freeze-thaw cycles are not recommended.
10. FBS should only be added to the media for initial plating and short term culture (up to four days). For long term culture, serum should not be added after day four.

## Coating Plates

Primary retinal cells need an appropriate substrate to adhere and survive. The preferred substrate is Matrigel. Poly-L-Lysine or Poly-D-Lysine can also be used alone to coat the cell culture plastic ware 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 Poly-L-Lysine or Poly-D-Lysine are available on our website at [www.lonza.com](http://www.lonza.com). For coating plates with Matrigel, do the following:

1. Thaw the bottle of Matrigel overnight at 4°C on ice.
2. Using pre-cooled pipette tips, transfer 5 ml of the Matrigel to a sterile, pre-cooled 50 mL centrifuge tube.
3. Add 45 ml of PNGM™ medium (not containing serum) or standard MEM (not containing serum) to the Matrigel and mix well.
4. Aliquot the diluted Matrigel in 1 mL aliquots into sterile, pre-cooled 1.5 mL Eppendorf® tubes and store immediately in a -20°C freezer. Avoid multiple freeze thaws. Do not store in a frost-free freezer.
5. When ready to plate, thaw an aliquot of the diluted Matrigel at 4°C on ice.
6. Using pre-cooled pipette tips, transfer enough of the diluted Matrigel to each well of a sterile, pre-cooled cell culture plate to sufficiently cover the growth surface of the well.

7. Incubate the plate at room temperature for at least one minute then aspirate the unbound Matrigel using a sterile pipette.
8. Incubate the plate in a humidified 37°C incubator with 5% CO<sub>2</sub> for 30 minutes and plate cells immediately afterwards.

## Thawing of Cells / Initiation of Culture Process

1. DAY 1: Prepare twenty wells of a 96-well plate or four wells of a 24-well plate for each rat retinal cell cryovial using the provided plate coating guidelines.
2. Wipe cryovial with ethanol or isopropanol before opening. Prior to thawing the cells, place the cryovial in a sterile field and briefly twist the cap a one-fourth turn to relieve pressure, and then re-tighten. Keep the time between removing the vial from the liquid nitrogen tank and placing into a pre-heated water bath as short as possible, quickly thaw the cryovial in a 37°C water bath, being careful not to submerge the entire vial. Wipe cryovial with ethanol or isopropanol before opening. Watch your cryovial closely; when the last sliver of ice melts, remove it. Do not submerge it completely. Thawing the cells for longer than 2 minutes results in less than optimal results.
3. Centrifugation should not be performed to remove cells from cryoprotectant cocktail. This action is more damaging than the effects of residual DMSO in the culture.
4. Remove vial from the water bath and disinfect the outside of the vial by wiping with 70% ethanol or isopropanol. Place in a laminar flow hood. Proceed with the next step immediately after thawing.
5. Gently transfer 0.5 ml cells into a 15 ml centrifuge tube and immediately add 3.5 ml pre-warmed medium containing 5% heat inactivated FBS drop-wise onto cells, while rotating the tube by hand. This should take approximately two 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 cell first by pipetting slowly up and down once, then aliquot the cells into the appropriate vessels.
6. Mix cell suspension by inverting the tube carefully, twice. IMPORTANT: do not vortex the cells.

7. Transfer cell suspension to appropriate well plate. See chart below for recommended volumes of medium.
8. Incubate the cells for four hours in a 37°C, 5% CO<sub>2</sub> incubator.
9. Remove the medium from the cells leaving a small volume to ensure the cells do not dry out and add fresh, pre-warmed medium. It is recommended to use medium containing 5% FBS for the initial three to four days after plating.
10. Incubate the cells at 37°C with 5% CO<sub>2</sub>.
11. DAY 4: Remove the medium from the cells leaving a small volume to ensure the cells do not dry out and add fresh, pre-warmed medium without FBS.

Recommended Seeding Volume after Diluting 0.5 ml Cell Suspension in 3.5 ml Fresh Culture Media	Plating Format	Number of Wells to Plate
200 µl/well	96-well plate	20 wells
1 ml/well	24-well plate	4 wells

## Maintenance after Plating

1. After initial medium change on DAY 4, replace 50% of the serum-free growth medium every three to four days for a longer term culture.
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 media loss due to evaporation should be taken into consideration. Add additional media whenever necessary.

## Quality Control

The cryopreserved cells are batch-tested for viability based on counts of ganglion cells compared to freshly prepared retinal cells at day seven in culture. The cells test negative for mycoplasma and bacteria. Additional molecular and immunochemical testing for quality is done following conditions that mimic shipping.

## Ordering Information

### Rat Retinal Cells (Pooled)

Cat. No.	Product	Description
R-Ret-508	Rat retinal cells, cryopreserved	≥ 0.2 x 10 <sup>6</sup> viable cells in a 0.5 ml cell suspension

### Primary Neuron Growth Media (Sold Separately):

Cat. No.	Product	Description
CC-4461	PNGM™ BulletKit™ Medium	200 ml PNBMT™ Basal Medium plus CC-4462 SingleQuots™ Kit to formulate PNGM™ Medium (growth medium)
CC-3256	PNBM™ Basal Medium	Primary neuron basal medium (200 ml)
CC-4462	PNGM™ SingleQuots™ Kit	Formulates 200 ml of PNBMT™ Basal Medium to PNGM™ Growth Medium; contains NSF-1, 4.0 ml; L-glutamine, 2.0 ml; GA, 0.2 ml
14-503E	FBS, heat inactivated	100 ml

## Product Warranty

Cultures have a finite lifespan *in vitro*.

Lonza guarantees the performance of its cells in the following manner only if Clonetics™ Media and Reagents are used exclusively and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete cell system.

1. Clonetics™ Rat Retinal Cells are assured to be viable and functional when thawed and maintained properly.
2. Clonetics™ Rat Retinal Cells are cryopreserved immediately after isolation without culturing prior to cryopreservation. Routine characterization of retinal cells includes positive immunostaining for neuron specific class III β-tubulin (Tuj-1), specific neuronal protein gene product (PGP 9.5), ganglion cell marker, Thy1.1, and GFAP. Lonza guarantees rat retinal cells will express the markers described when plated out of cryopreservation onto Matrigel.

When placing an order or to contact Scientific Support, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics™ Products, refer to the Lonza website

or the current Lonza catalog. To obtain a catalog, additional information or to speak with Scientific Support, you may contact Lonza by web, e-mail, telephone, fax or mail (See page 1 for details).

**THESE PRODUCTS ARE FOR RESEARCH USE ONLY.** Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or *in vitro* 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.

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