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Rat Cardiac Myocytes
Instructions for use

I. 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 SingleQuots™ Kit at ≤20°C in a freezer that is not self-defrosting. Once thawed, SingleQuots™ Kit should be stored at 2° - 8°C and added to basal medium within 72 hours. After SingleQuots™ 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.

II. Preparation of media

The recommended medium for the rat cardiac myocytes is the RCGM BulletKit™. The BulletKit™ contains a 200 mL bottle of Rat Cardiac Myocyte Basal Medium (RCBM) and RCGM SingleQuots™ Kit. Bromodeoxyuridine (BrdU, sold separately) must be reconstituted in basal media, aliquoted, frozen and then added to the media as needed immediately before each use.

1. Thaw the SingleQuots™ Kit 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 Rat Cardiac Myocyte Growth Media (RCGM Medium), transfer the contents of the RCGM SingleQuots™ Kit (Catalog No. CC-4516 containing Horse Serum, Fetal Bovine Serum [FBS], and Gentamicin/Amphotericin-B [GA]) to RCBM 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. RCGM Medium must be further supplemented with BrdU (sold separately) to prevent the overgrowth of fibroblasts. To prepare 40 mM BrdU Stock Solution, dissolve 12.3 mg of 5-bromo-2'-deoxyuridine (BrdU; Sigma Catalog

No. B5002 or equivalent) in 1 mL of RCBM Basal Media.

7. Transfer the desired volume of RCGM Growth Medium to a sterile secondary container and add 40 mM BrdU Stock Solution to a final concentration of 200 µM. For Example: Add 25.00 µL of 40 mM BrdU Stock Solution to 5 mL of RCGM Growth Medium.

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 BrdU is not recommended.**

8. Aliquot remaining 40 mM BrdU Stock Solution at desired volumes and store at 4°C for up to one month or -20°C for up to one year.
9. Thaw individual 40 mM BrdU Stock Solution aliquots as needed to prepare fresh media. Additional freeze-thaw cycles are not recommended.

III. Coating plates

Primary rat cardiac myocytes need an appropriate substrate to adhere and survive. The preferred substrate is nitrocellulose. For coating plates with nitrocellulose, do the following:

1. Prepare a Nitrocellulose Stock Solution by dissolving 1 cm^2 nitrocellulose paper (Bio-Rad Catalog No. 162-00113 or equivalent) in 10 mL of methanol and filter through a 0.22 µm syringe filter.
2. Aliquot the Nitrocellulose Stock Solution in 0.5 mL aliquots into sterile, 1.5 mL Eppendorf tubes and store at room temperature.
3. When ready to plate, dilute the Nitrocellulose Stock Solution at 1:10 in methanol to create the Nitrocellulose Working Solution. For example: Add 100 µL of Nitrocellulose Stock Solution to 900 µL of methanol.
4. Transfer 10 µL of the Nitrocellulose Working Solution per well of a 24-well plate, 3 µL of the Nitrocellulose Working Solution per well of a 96-well plate, or 8 µL of the Nitrocellulose Working Solution per glass coverslip.
5. Incubate the plate at room temperature for at least 5 minutes until the solution has completely dried and plate cells immediately afterwards.

IV. Thawing of cells / initiation of culture process

1. DAY 1: Prepare twenty-six wells of a 96-well plate or ten wells of a 24-well plate for each rat cardiac myocyte 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 1.0 mL cells into a 15 mL centrifuge tube and immediately add pre-warmed medium (without BrdU) drop-wise onto cells, while rotating the tube by hand. If plating cells in a 96-well plate, add 4.3 mL of media for a total volume of 5.3 mL. If plating cells in a 24-well plate, add 9.0 mL of media for a total volume of 10.0 mL. 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. Place the cells very slowly in the center of the well. To evenly distribute the cells over the well surface, gently shake the plate in a cross motion (once left, once right, once up and once down) with the plate on a flat surface.
9. Incubate the cells for four hours in a 37°C, 5% CO_2 incubator.
10. Remove 80% the medium from the cells and replace with fresh, pre-warmed medium containing 200 µM BrdU.
11. Incubate the cells at 37°C with 5% CO_2.

NOTE: Cell death will be observed; cultivation of cells should be continued.

12. DAY 3: Remove 50% the medium from the cells and replace with fresh, pre-warmed medium containing 200 µM BrdU.
13. For a longer period of cultivation, remove 50% the medium from the cells and replace with fresh, pre-warmed medium containing 200 µM BrdU every 3 days.

Plating format	Volume of media to add to 1.0 mL cell suspension	Recommended seeding volume after diluting	Number of wells to plate
96-well plate	4.3 mL	200 µL/well	26 wells
24-well plate	9.0 mL	1 mL/well	10 wells

V. Maintenance after plating

1. After initial medium change on DAY 3, replace 50% of the growth medium containing 200 µM BrdU every three 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 containing 200 µM BrdU 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.

VI. Quality control

The cells test negative for mycoplasma and bacteria. Additional molecular and immunochemical testing for quality is done following conditions that mimic shipping.

VII. Ordering information

Rat Cardiac Myocytes (pooled)

Cat. no.	Product	Description
R-CM-561	Rat cardiac myocyte (CM) cells, cryopreserved	4 million cells in a 1.0 mL cell suspension

Rat Cardiac Growth Media (sold separately):

Cat. no.	Product	Description
CC-4515	RCGM BulletKit™ Medium	200 mL RCBM Basal Medium plus CC-4516 SingleQuots™ Kit to formulate RCGM Medium (growth medium)
CC-3275	RCBM Basal Medium	Rat Cardiac Basal Medium (200 mL)
CC-4516	RCGM SingleQuots™ Kit	Formulates 200 mL of RCBM Basal Medium to RCGM Growth Medium; contains Horse Serum, 15.0 mL; FBS, 15.0 mL; GA, 0.2 mL

VIII. Product warranty

Cultures have a finite lifespan *in vitro.*

Lonza guarantees the performance of its cells in the following manner only if Lonza 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. Rat Cardiac Myocytes are assured to be viable and functional when thawed and maintained properly.
2. Rat Cardiac Myocytes are cryopreserved immediately after isolation without culturing prior to cryopreservation. Routine characterization of cardiac myocytes includes positive immune-staining for actinin and positive testing for functional syncytium. Lonza guarantees rat cardiac myocytes will express the markers described when plated out of cryopreservation onto nitrocellulose.

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 Lonza 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.