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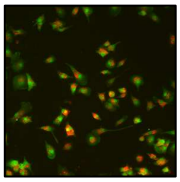

Clonetics™ Rat Microglia Cells

R-G - Instructions for Use

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℃. 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.
1. Upon arrival, store basal medium at 4℃, protected from the light. Store heat-inactivated fetal bovine serum and penicillin/streptomycin reagents at -20℃ in a freezer that is not self-defrosting. Store D-Glucose at room temperature. Once the media is supplemented with FBS, penicillin/streptomycin, and D-Glucose, it may be stored for up to 4 weeks at 4℃. 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 microglia cells is DMEM (Lonza Catalog No. 12-604F or equivalent) supplemented with 10% heat-inactivated fetal bovine serum (Sigma Catalog No. F4135 or equivalent), 4.5 g/L D-glucose (Sigma Catalog No. G8769 or equivalent), and 1% penicillin/streptomycin (Lonza Catalog No. 17-602E or equivalent).

1. Thaw the penicillin/streptomycin and heat-inactivated fetal bovine serum at room temperature or overnight in a 2-8℃ refrigerator.
2. Decontaminate external surfaces of all vials and the medium bottle with ethanol or isopropanol.
3. Transfer 176 ml of the DMEM to a sterile 250 ml Nalgene bottle.
4. To formulate Rat Microglial Cell Growth Media, transfer 20.0 ml of heat inactivated fetal bovine serum, 2.0 ml of penicillin/streptomycin, and 2.0 ml of D-glucose into the 176 ml DMEM bottle with a pipette. Use the table below as a guideline:

Medium / Supplement Description	Vendor / Catalog Number	Volume Required	Final Concentration / Percentage
DMEM	Lonza: 12-604F	176 m	88%
Heat Inactivated Fetal Bovine Serum	Sigma: F4135	20 ml	10%
100x Penicillin / Streptomycin Solution	Lonza: 17-602E	2.0 ml	100 units potassium penicillin and 100 µg streptomycin sulfate per ml
45% D-Glucose Solution	Sigma: G8769	2.0 ml	4.5 g/L (in addition to 4.5 g/L in basal media for final D-glucose concentration of 9.0 g/L)

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.

5. Aliquot remaining heat inactivated fetal bovine serum and penicillin/streptomycin solution at desired volumes and store at -20°C.

Thawing of Cells / Initiation of Culture Process

1. DAY 1: 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.
2. 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.
3. 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.
4. Mix cell suspension by gently pipetting once up and down. IMPORTANT: do not vortex the cells.
5. Add 1,080 µl of pre-warmed medium Rat Microglial Cell Growth Media drop-wise directly into the vial of cells, while rotating the vial 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 gently pipetting once up and down. IMPORTANT: do not vortex the cells.
7. Transfer cell suspension to appropriate well plate. 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.

8. Incubate the cells for twenty-four hours in a 37°C, 5% CO_2 incubator.
9. Very gently, remove 50% the medium from the cells and replace with fresh, pre-warmed medium.
10. Incubate the cells at 37°C with 5% CO_2.

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

Plating Format	Volume of Media to Add to 0.25 ml Cell Suspension	Recommended Seeding Volume after Diluting	Number of Wells to Plate
96-well plate	1080 µl	100 µl/well	13 wells

Quality Control

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 Microglia Cells (Pooled)

Cat. No.	Product	Description
R-G-535	Rat Microglia Cells, cryopreserved	≥2 million viable cells in a 0.25 ml cell suspension

Rat Microglia Growth Media (Sold Separately):

Cat. No.	Product	Description
12-604F	DMEM	Dulbecco's Modified Eagle's Medium with 4.5 g/L glucose, with L-glutamine, 500 ml
17-602E	Penicillin-Streptomycin Mixture	Contains 10,000 units potassium penicillin and 10,000 µg streptomycin sulfate per ml in 0.85% saline, 100 ml

Heat Inactivated Fetal Bovine Serum and 45% D-glucose are also necessary to create complete rat microglia cell growth media and must be purchased separately.

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 Microglia Cells are assured to be viable and functional when thawed and maintained properly.
2. Clonetics™ Rat Microglia Cells are cryopreserved immediately after isolation without culturing prior to cryopreservation. Routine characterization of microglia cells includes positive immunostaining for microglia specific Tomato Lectin (TL) and IBA1. Lonza guarantees rat microglia cells will express the markers described when plated out of cryopreservation.

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).