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Clonetics™ normal human astrocytes

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 clinical or *in vitro* procedures.

WARNING: CLONETICS™ AND POIETICS™ PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, hepatitis B virus and hepatitis C virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, hepatitis B virus, and hepatitis C virus. Testing can not offer complete assurance that HIV-1, hepatitis B virus, and hepatitis C virus are absent. All human sourced products should be handled at the biological safety level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 5th edition. If you require further information, please contact your site safety officer or scientific support.

Unpacking and storage instructions

1. Check all containers for leakage or breakage.
2. For cryopreserved cells – remove cryovials from the dry ice packaging and immediately place into liquid nitrogen storage. Alternatively, thaw and use the cells immediately. If no dry ice remains, please contact customer service.
3. For proliferating cells – swab down the flask of proliferating cells with 70% ethanol or isopropanol, then place the flask in 37°C, 5% CO_2, humidified incubator and allow to equilibrate for 3 to 4 hours. After cells have equilibrated, remove shipping medium from the flask and replace with fresh medium.
4. AGM™ BulletKit™ instructions: Upon arrival, store astrocyte basal medium (ABM™) at 2°C to 8°C and SingleQuots™ at -20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 2°C to 8°C and added to ABM™ within 72 hours of receipt. After SingleQuots™ are added to basal medium, use within 1 month. Do not re-freeze.
5. ReagentPack™ subculture reagents are sterile-filtered and then stored at -20°C until shipment. Subculture reagents may thaw during transport. They may be refrozen once. If you plan to use within 3 days, store at 2°C to 8°C. Trypsin/EDTA solution has a limited shelf life or activation at 2°C to 8°C. If, upon arrival, trypsin/EDTA is thawed, immediately aliquot and refreeze at -20°C. We recommend that the HEPES-BSS and the trypsin neutralizing solution be stored at 2° to 8°C for no longer than one month.

   Note: To keep trypsin/EDTA fresh and active after thawing, you may aliquot it into sterile centrifuge tubes and re-freeze at -20°C.

Using media or reagents other than what's recommended will void the cell warranty. Please contact scientific support if you need help selecting media and/or reagents.

Preparation of media

For a BulletKit™, perform the following steps:

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the entire amount to the basal medium with a pipette.
3. Rinse each cryovial with the medium. It may not be possible to recover the entire volume listed for each cryovial. Small losses, even up to 10%, should not affect the cell growth characteristics of the supplemented medium.
4. Transfer the label provided with each kit to the basal medium bottle being supplemented. Use it to record the date and amount of each supplement added. We recommend that you place the completed label over the basal medium label, allowing for the basal medium lot number and expiration date to be visible.
5. Record the new expiration date on the label based on the shelf life.

Note: If there is concern that sterility was compromised during the supplementation process, the entire newly prepared growth medium may be refiltered with a 0.2 μm filter to assure sterility. Routine refiltration is not recommended.

Thawing of cells / initiation of culture process

1. The recommended seeding density for NHA is 5,000 cells/cm^2.
2. To set up cultures calculate the number of vessels needed based on the recommended seeding density of 5,000 cells/cm^2 and the surface area of the vessels being used. Do not seed cells into a well plate immediately out of cryopreservation. Add the appropriate amount of medium to the vessels (1 ml/5 cm^2) and allow the vessels to equilibrate in a 37°C, 5% CO_2, humidified incubator for at least 30 minutes.
3. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, then retighten. Quickly thaw the cryovial in a 37°C water bath by gently swirling the vial only until the ice disappears. 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.
4. Resuspend the cells in the cryovial and dispense them into the preincubated culture vessels at the calculated seeding density. Gently rock the culture vessel to evenly distribute the cells and return to the incubator.
5. Centrifugation should not be used to remove cells from cryopreservation medium. This action is more damaging than the effects of residual DMSO in the culture.

Subculturing

The following instructions are for a 25 cm^2 flask. Adjust all volumes accordingly for other size flasks.

Note: Lonza warrants its Clonetics™ cells only if Lonza subculturing reagents are used. The recommended subculturing reagents for these cells are trypsin/EDTA (CC-5012), trypsin neutralizing solution (CC-5002), and HEPES buffered saline solution (CC-5022). These reagents can be purchased individually or together as part of the ReagentPack™ subculture reagents (CC-5034).

Preparation for subculturing the first flask:

1. Subculture the cells when they are 70-80% confluent and contain many mitotic figures throughout the flask.
2. For each 25 cm^2 of cells to be subcultured:
   - Thaw 2 ml of trypsin/EDTA and allow to come to room temperature.
   - Allow 7-10 ml of HEPES buffered saline solution (HEPES-BSS) to come to room temperature.
   - Allow 4 ml of trypsin neutralizing solution (TNS) to come to room temperature.
3. Remove growth medium from 2°C to 8°C storage and allow to warm to room temperature.
4. Prepare new culture flasks by adding pre-warmed medium at a volume of 1 ml/5 cm^2 of surface area, label with cell strain, lot number, passage and date. Place the flasks in the incubator until cells are ready to be seeded.

In a sterile field:

1. Aspirate the medium from 1 flask.
2. Rinse the cells with 5 ml of room temperature HEPES-BSS. DO NOT forget this step. The medium contains trypsin inhibitors such as calcium or serum proteins.
3. Aspirate the HEPES-BSS from the flask.
4. Cover the cells with 2 ml of trypsin/EDTA solution.
5. Place the flask in the 37°C incubator for 3 to 4 minutes.
6. Allow the trypsinization to continue until approximately 90% of the cells have rounded up. Examine the culture flask under the microscope to determine the extent of the detachment.
7. At this point, rap the edge of the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, return the flask to the incubator for 30 seconds to 1 minute.
8. After the cells have detached, neutralize the trypsin in the flask with 4 ml of room temperature trypsin neutralizing solution.
9. If a majority of the cells do not detach within 5 minutes, the enzyme activity of the trypsin has been compromised by low temperature or an overextended shelf life. Proceed to harvest the cells from the flask as described above, and either re-trypsinize with fresh, warm trypsin/EDTA solution or rinse with trypsin neutralizing solution, add fresh, warm medium to the flask and return to the incubator until fresh trypsinization reagents are available.
10. Quickly transfer the detached cells to a sterile 15 ml centrifuge tube.
11. Rinse the flask with a final 2 ml of HEPES-BSS to collect residual cells, and add this rinse to the centrifuge tube.

12. Examine the flask under the microscope to make sure the harvest was successful by observing the number of cells remaining. This should be less than 5%.
13. Centrifuge the cell suspension at 160 x g to 200 x g for 5 minutes at 2°C to 8°C.
14. Aspirate the supernatant and resuspend the cell pellet in 2 ml of AGM™.
15. Determine cell count and viability using a hemacytometer and trypan blue.
16. Calculate the volume of the cell suspension needed to seed the flask at a density of 5,000 cells/cm^2 and add the appropriate volume of cell suspension to a tissue culture flask or dish (pre-filled at 1 ml/5cm^2 of surface area with pre-warmed medium). If seeding into well plates at this time, the recommended density is 10,000 cells/cm^2. Mix gently to evenly distribute the cells and return the flask to the incubator.

Maintenance

1. Change the growth medium the day after seeding and every other day thereafter. As the cells become more confluent, increase the volume of media as follows: under 25% confluence then feed cells 1 ml per 5 cm^2, 25-45% confluence then feed cells 1.5 ml per 5 cm^2, over 45% confluence then feed cells 2 ml per 5 cm^2.
2. Warm an appropriate amount of medium to 37°C in a sterile container. Remove spent medium from flask and replace it with the warmed, fresh medium and return the flask to the incubator.
3. Avoid repeated warming and cooling of the medium. If the entire contents are not needed for a single procedure, transfer and warm only the required volume to a sterile secondary container.

Ordering information

Cryopreserved cells (single donor)		
CC-2565	NHA	≥1,000,000 cells
Proliferating cells		
CC-2665	NHA	T-25 flask
CC-0297	NHA	T-75 flask
CC2565T150	NHA	T-150 flask
CC2565T225	NHA	T-225 flask
Proliferating cells in preseeded plates		
CC-2565W6	NHA	6 wells
CC-2565W12	NHA	12 wells
CC-2565W24	NHA	24 wells
CC-2565W48	NHA	48 wells
CC-0093	NHA	96 wells

Related products

Astrocyte medium (must be purchased separately):

CC-3186	AGM™ BulletKit™	Kit which contains a 500 ml bottle of ABM™, (CC-3187) and AGM™ SingleQuots™ (CC-4123)
CC-3187	ABM™	Astrocyte basal medium (no growth factors) (500 ml)
CC-4123	AGM™ SingleQuots™	Supplements for a complete growth medium, developed especially for NHA (CC-2565)

Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza warrants its cells in the following manner only if Clonetics™ media and reagents are used.

1. Clonetics™ NHA cryopreserved cultures are assured for experimental use for at least 10 population doublings.
2. Clonetics™ NHA proliferating cultures are assured for experimental use for at least 5 population doublings.
3. Additional population doublings and subcultures are possible, but growth rate, biological responsiveness and function deteriorate with subsequent passage.
4. To avoid the loss of your cells and forfeiture of your warranty, subculture cells before they reach 80% confluence.

Quality control

HIV-1, hepatitis B and hepatitis C are not detected for all donors and/or cell lots. Routine characterization of NHA includes positive immunofluorescence staining for glial fibrillary acid protein (GFAP) in the first passage out of cryopreservation; GFAP expression decreases with passaging. For detailed information concerning QC testing, please refer to the certificate of analysis.

When placing an order or for 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 our current catalog. To obtain a catalog, additional information or technical support you may contact Lonza by web, e-mail, telephone, fax or mail.