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Document # INST M-AsM-430-CD1-5 08/11

Cryopreserved mouse 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 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. Check all containers for leakage or breakage.
2. Store in liquid nitrogen. Do NOT store cells at -80℃. The cells are extremely temperature-sensitive and should be transferred to liquid nitrogen immediately upon arrival. For transportation of vials use dry ice or a liquid nitrogen container. When transporting cells on dry ice make sure that vials are completely covered by dry ice.

Recommended media

The recommended media for the mouse astrocyte cells is the astrocyte growth medium BulletKit™. The BulletKit™ contains a 500ml bottle of ABM™ (astrocyte basal medium) and AGM™ SingleQuots™.

Preparation of media

To prepare the BulletKits™, 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

NOTE: For optimal performance, positively charged plasticware is recommended.

1. DAY 1: Remove a vial of cells from liquid nitrogen and place in a water bath pre-heated to 37℃. **IMPORTANT: do not 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 2½ minutes, 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 of 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.

4. Mix the 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.
6. Incubate the cells for 6 hours at 37℃ in 5% CO_2 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. On day 7, change 50% of the media to fresh astrocyte growth media (warmed to 37℃). Additional media changes should be performed every 4-5 days.
9. When the cells are confluent, they can be trypsinized and re-plated.

Volume of astrocyte growth media (AGM™)	Plating format
4.5 ml	0.5 ml cell suspension
5 ml cells + 3 ml additional AGM™	50 ml flask (i.e. T-25 flask)
1 ml/well	24-well plate
2 ml/dish	35mm petri dish

Subculturing

1. Trypsinize the cells using 0.25% trypsin.
2. Count the number of cells using a hemocytometer.
3. Centrifuge the cells a 300 x g for 10 minutes.

Maintenance

1. After the initial media change on day 7, replace 50% of the growth media every 4-5 days.
2. Warm an appropriate amount of medium to 37℃ 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.

Ordering information

M-AsM-330	Mouse C57 mixed astrocytes	≥1,000,000 cells
M-AsM-430	Mouse CD1 mixed astrocytes	≥1,000,000 cells

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

Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza guarantees cell performance only when the approved media and supplements are used. Please contact scientific support for further information on the approved media and supplements.

Quality control

The cells test negative for mycoplasma and bacteria. The astrocytes are batch tested for growth characteristics and morphology (GFAP).

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, please refer to the Lonza website or our current catalog. To obtain a catalog, additional information or scientific support you may contact Lonza by web, e-mail, telephone, fax or mail.