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Clonetics™ mouse embryo fibroblast cells

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. Cells should be stored 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. Cells should be transported on dry ice or in a liquid nitrogen container. When transporting the cells on dry ice make sure that the vials are **completely** covered.

Preparation of medium

The recommended media for the MEF cells is Lonza DMEM high glucose containing 10% FBS.

1. Thaw the FBS and pen/strep at room temperature.
2. Decontaminate the external surfaces of all supplement vials and the media bottle with ethanol or isopropanol.
3. Add the required amount to basal medium with a pipette:
   - 89.9 ml Dulbecco's modified eagle medium (DMEM)
   - 10 ml fetal bovine serum (heat inactivated)
   - 0.1 ml pen/strep

NOTE: If there is a concern that sterility was compromised during the process, the medium may be filtered with 0.2 µm filter to assure sterility. Routine refiltration is not recommended.

Thawing of cells / initiation of culture process

1. Day 1: Remove a vial of cells from liquid nitrogen and place in a water bath pre-heated to 37℃. Important: Do not centrifuge or vortex the cells, keep the time between removing the vial from liquid nitrogen tank and placing into the preheated water bath as short as possible.
2. After 2 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 the 1 ml cell suspension into a 15 ml centrifuge tube and immediately add pre-warmed medium containing 10% FBS drop-wise onto 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. Transfer cell suspension to the culture vessel at the recommended seeding density for MEF Cells which is 8000 cells/cm² - about 1.4 million cells in a T-175 flask, 0.6 million cells in a T-75 flask, 0.2 million cells in a T-25 flask, 6000 cells/well in a 48-well plate and 3000 cells/well in a 96-well plate.
5. Incubate cells at 37℃ in 5% CO_2 incubator for about 12-20 hours.
6. Remove the medium from cells and add fresh pre-warmed medium.
7. Change 75% of medium to fresh pre-warmed medium every 4-5 days.
8. When cells appear confluent, they can be trypsinized and re-plated.

Subculture:

The following instructions are for a T-25 flask. Adjust all volumes accordingly for other size flasks.

Preparation for subculturing

1. Subculture the cells when they are 80-90% confluent.
2. For each 25 cm² of cells to be subcultured:
   a. Thaw 2 ml of trypsin-Versene® and allow to come to room temperature.

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b. Allow 5 ml of HEPES buffered saline solution (HEPES-BSS) to come to room temperature.
c. Allow 4 ml of trypsin neutralizing solution (TNS) to come to room temperature.
d. Remove the growth medium from 4°C storage and allow to warm to room temperature.

Work in a laminar flow hood

1. Prepare T-25 culture flasks for inoculation by adding 5 ml growth medium and equilibrate them in the incubator at 37°C, 5% CO_2 for 30 minutes before inoculating the cells.
2. Aspirate medium from the growth flask and add 5 ml HEPES-BSS to rinse the cells.
3. Aspirate HEPES-BSS from the flask and cover the cells in the flask with 2 ml trypsin-Versene® (EDTA) mixture 1X solution 17-161.
4. Rock the flask to make sure all cells come into contact with the trypsin-Versene®.
5. Tighten the cap and place the flask into the incubator.
6. Remove the flask after 2 minutes and check for cell detachment under the microscope, if majority of cells are not detached, return to incubator and check cells every 2 minutes until 90% of the cells are rounded up (see step 7). Do not allow trypsin to stay on the monolayer longer than 10 minutes.
7. When most of the cells are rounded up, tap the flask against the palm of your hand to release the majority of the cells from the culture flask. If only a few cells detach, you may not have let them trypsinize long enough. Wait 30 seconds and tap again. Repeat the procedure until 90% of cells are detached. Do not try to get all cells detached by tapping them severely. This action may damage the cells.
8. After cells are released, neutralize the trypsin in the flask with 4 ml TNS solution.
9. Transfer the cell suspension to a 15 ml conical tube.
10. Centrifuge the cells at 300 x g for 5 minutes.
11. Perform a cell count and calculate the total number of cells and the volume of cell suspension needed to inoculate the flasks.
12. Dispense the calculated volume into prepared subculture flasks.
13. Place the culture flasks into 37°C, 5% CO_2 humidified incubator.

Maintenance

1. After initial media change on day 1, replace 75% of the growth medium every 4 to 5 days.
2. Warm an appropriate amount of medium to 37°C in a sterile container, remove 75% 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-Fb-481	Mouse embryonic fibroblasts	≥ 2 million cells in a 1 ml cell suspension
12-604F	DMEM, high glucose	500 ml
14-503E	FBS, US origin, heat inactivated	100 ml
17-603E	Penicillin-streptomycin mixture	100 ml
CC-5022	HEPES	100 ml
17-161E	Trypsin-Versene® mixture	100 ml
CC-5002	Trypsin neutralizing solution	100 ml

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 service you may contact Lonza by web, e-mail, telephone, fax or mail.

Product warranty

CULTURES HAVE A FINITE LIFESPAN *IN VITRO*. Lonza guarantees cell performance only when the approved media and supplements are used.

Quality control

The cells test negative for mycoplasma and bacteria. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation.

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