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Clonetics™ rat aortic smooth muscle 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 i*n 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.
2. Upon arrival, store basal medium at 4℃, protected from the light. Store heat-inactivated fetal bovine serum and GA-1000 reagents at -20℃ in a freezer that is not self defrosting. Once the media is supplemented with FBS and GA-1000, it may be stored for up to 4 weeks at 4℃.

Preparation of medium

The recommended medium for the rat aortic smooth muscle cells is DMEM:F12, (Lonza No. BE04-687Q) supplemented with 20% heat-inactivated fetal bovine serum (Lonza No. 14-503E) and 1/1000 GA-1000 (Lonza No. CC-4083).

To prepare 200 ml complete growth medium do the following:

1. Wipe DMEM:F12 bottle with ethanol or isopropanol.
2. Transfer 160 ml of the basal medium to a 250 ml sterile nalgene bottle.
3. Thaw the GA-1000 vial and heat-inactivated FBS bottle at room temperature.
4. Decontaminate the external surface of the supplement bottle and vial with ethanol or isopropanol.
5. Add 40 ml of heat-inactivated FBS and 0.2 ml GA-1000 into the 160 ml basal medium bottle. Aliquot and freeze remainder of FBS and GA-1000.

   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.

Use the table below as a guideline:

Medium/supplements	Amount needed
DMEM:F12 (BE04-687Q)	160 ml
HI FBS (14-503E)	40 ml
GA-1000 (CC-4083)	0.2 ml

Thawing of sells / initiation of culture process

1. The recommended seeding from the cryo vial is 5000 cells/cm².
2. To set up the culture, add the appropriate amount of medium to the vessels (1ml/5cm²) and allow the vessels to equilibrate in a 37℃ 5% CO_2 humidified incubator for 30 minutes.
3. 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. Swirl the vial in the water bath and remove as soon as the ice has disappeared (about 1-1.5 minutes). **Do not allow the vial to remain in the water bath any longer than it takes to thaw the cells**.
4. 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.

5. Mix the cell suspension by inverting the tube carefully, twice. Important: do not vortex the cells.
6. Perform a viability count and calculate the volume of cell suspension required to seed 5000 cells/cm².
7. Transfer cell suspension to appropriate flasks, dishes or well plates.
8. Incubate the cells at 37°C in 5% CO_2 humidified incubator.
9. Change the growth medium the day after seeding to remove residual DMSO and unattached cells, then every other day thereafter. Increase the volume of medium as the culture becomes more confluent. Use the table below as a guideline:

If the culture is:	Then feed with:
Under 25% confluent	1 ml per 5 cm²
25 to 45% confluent	1.5 ml per 5 cm²
Exceeding 45% confluence	2 ml per 5 cm²

NOTE: Warm media to room temperature before feeding cells. Avoid warming and cooling of whole bottle, only warm the volume of medium you need to feed the cells.

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 (about 3 to 5 days).
2. For each 25 cm² of cells to be subcultured:
    a. Thaw 2 ml of trypsin/EDTA and allow to come to room temperature.
    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 min 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/EDTA.
4. Rock the flask to make sure all cells come into contact with the trypsin/EDTA Solution.
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 the majority of cells are not detached, return to incubator and check cells every minute until 90% of the cells are rounded up (see step 7). Do not allow trypsin to stay on the monolayer longer than 5 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.
14. After the first passage out of cryopreservation, cells proliferate faster and the number of cells seeded can be lowered to 3000 cells/cm².

Ordering information

R-ASM-580	Rat aortic smooth muscle cells	≥0.5 million cells in a 0.5 ml cell suspension
BE04-687Q	DMEM:F12	1 L
14-503E	FBS, heat inactivated	100 ml

CC-4083	GA-1000	5 ml
CC-5034	ReagentPack™	Contains 100 ml each: HEPES, trypsin/EDTA and trypsin neutralizing solution.

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 cryopreserved cells are batch-tested for viability, seeding efficiency, doubling time, and total population doublings. Cell purity is assessed by immunostaining with alpha-smooth muscle actin and VE-cadherin. The cells test negative for mycoplasma and bacteria. For detailed information concerning QC testing, please refer to the certificate of analysis.