Clonetics® Human Sertoli Cells
Instructions for Use

Safety Statements
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-1, 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, 1999. 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. BulletKit® Instructions: Upon arrival, store Basal Medium at 2°C - 8°C and SingleQuots® at minus 20°C in a freezer that is not self-defrosting. If thawed upon arrival, growth factors can be stored at 2°C - 8°C and added to basal medium within 72 hours of receipt. After SingleQuots® are added to basal medium, use within one month. Do not re-freeze SingleQuots.

Using media 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 SeGM™ Media
Perform the following steps in a sterile field.

For the SeGM™ 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 (Avoid covering the basal medium lot # and expiration date) to avoid confusion or possible double supplementation.
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 Sertoli Cells is 4,000 to 5,000 cells/cm².
2. To set up cultures, calculate the number of vessels needed based on the recommended seeding density and the surface area of the vessels being used. Add the appropriate amount of medium to the vessels (1 ml/5 cm²) and allow the vessels to equilibrate in a 37°C, 5% CO₂, humidified incubator for at least 30 minutes. Do not seed cells into a well plate directly out of cryopreservation.
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 being careful not to submerge the entire vial. 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 using a micropipette, dispense cells into the prepared culture vessel(s). Gently rock the culture vessel(s) to evenly distribute the cells and return to the incubator.

5. 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.

Subculturing
The following instructions are for a 75 cm² flask. Adjust all volumes accordingly for other size flasks.

Preparation for subculturing the first flask:
1. Subculture the cells when they are 70 to 80% confluent and contain mitotic figures throughout the flask.
2. For each 75 cm² of cells to be subcultured, the following reagent volumes are required at room temperature:
   - 6 ml of Versene®
   - 4 ml of Trypsin/EDTA (CC-5012)
   - 15 ml of Phosphate Buffered Saline Solution (PBS)
   - 8 ml of Trypsin Neutralizing Solution (TNS)
   - 15 ml of SeGM™
3. Subculture one flask at a time. All flasks succeeding the first flask will be subcultured following an optimization of this protocol based on calculated cell count, cell viability, and seeding density.

In a sterile field:
1. Aspirate the medium from one flask.
2. Rinse the cells with 10 ml of room temperature PBS (without calcium or magnesium). DO NOT forget this step. The medium contains complex proteins and calcium that can neutralize trypsin.
3. Aspirate the PBS from the flask.
4. Cover the cells with 6 ml of Versene® Solution and incubate at 37°C for 3 to 5 minutes.
5. Examine the cell layer microscopically. Approximately 60-80% of the cells should be lifting off the surface of the flask.
6. At this point, rap the flask against the palm of your hand to release the majority of cells from the culture surface. If only a few cells detach, add 4 ml of trypsin-EDTA to the flask and return to the incubator (37°C) for an additional 2-3 minutes. During the 3 minute incubation, take out the flask and rap again. If cells still do not detach, wait and rap every 30 seconds thereafter.
7. If trypsin is being used and after cells are released, neutralize the trypsin in the flask with 8 ml of room temperature Trypsin Neutralizing Solution (TNS).
8. Quickly transfer the detached cells to a sterile 50 ml centrifuge tube.
9. Rinse the flask with a final 5 ml of PBS to collect residual cells, and add this rinse to the centrifuge tube.
10. Examine the harvested flask under the microscope to ensure that the harvest was successful by looking at the number of cells left behind. This should be less than 5%.
11. Centrifuge the harvested cells at 220 x g for 5 minutes.
   a. Aspirate most of the supernatant, except for 100-200 μl.
   b. Flick the tube with your finger to loosen the pellet.
12. Dilute the cells to a final volume of 2 to 3 ml of growth medium and note the total volume of the diluted cell suspension.
13. Determine cell count and viability using a hemacytometer and Trypan Blue. Make a note of your cell yield for later use.
14. If the cell suspension is too dense to obtain an accurate count, dilute a sample of the suspension, record the dilution factor and re-count the cells.
15. Use the following equation to determine the total number of viable cells.

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\text{Total # of Viable Cells} = \frac{\text{Total cell count} \times \text{percent viability}}{100}
\]

16. After determining the cell yield and preferred seeding density, i.e., 4,000 or 5,000 per cm², calculate the number of flasks to be seeded. Label each flask with the passage number, lot number and date.
17. Add growth medium to the new flask(s), using 1 ml of growth medium for every 5 cm² of culture area, e.g., 15 ml per 75 cm² flask.
18. After mixing the cells with a 5 ml pipet to ensure a uniform suspension, dispense the calculated volume of cell suspension into each prepared subculture flask.
19. If not using vented caps, loosen caps of flasks. Place the new culture vessels into a 37°C humidified incubator with 5% CO₂.

Maintenance
1. Change the growth medium the day after seeding and every 3 – 4 days thereafter, using 1 ml of fresh media per 5 cm² of culture area, e.g., 15 ml per 75 cm² flask.
2. Warm the growth medium to 37°C. Remove the spent medium and replace it with warmed, fresh medium and return the flask(s) to the incubator.

3. Note: 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 in a sterile secondary container.

Related Products

Sertoli Cell Growth Medium  
(Must be purchased separately):

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>00191053</td>
<td>SeGM™ BulletKit® Kit which contains a 500 ml bottle of SeBM™ (00191051) and SeGM™ SingleQuots® (00191052).</td>
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<tr>
<td>00191051</td>
<td>SeBM™ Sertoli Cell Basal Medium (no growth factors) (500 ml)</td>
</tr>
<tr>
<td>00191052</td>
<td>SeGM™ SingleQuots® Supplements (FBS and gentamicin/ amphotericin-B)</td>
</tr>
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</table>

Subculturing Reagents  
(Must be purchased separately):

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>17-711E</td>
<td>Versene® [EDTA] 0.02% (100 ml)</td>
</tr>
<tr>
<td>CC-5012</td>
<td>Trypsin/EDTA (100 ml)</td>
</tr>
<tr>
<td>CC-5002</td>
<td>Trypsin Neutralizing Solution (TNS) (100 ml)</td>
</tr>
<tr>
<td>17-516F</td>
<td>PBS w/o Ca²⁺ or Mg⁺ (500 ml)</td>
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Limited Use License

Sertoli Cells are produced for Lonza by MandalMed Inc. and are subject to the following limited use license:

The included biological material, including progeny and derivatives, (collectively referred to as “Material”) is licensed to you under specific terms. You are responsible for ensuring that the terms of the license agreement are met.

GRANTS OF LICENSE: Lonza grants you a nontransferable, nonexclusive license to use the Material for research.

NOT FOR HUMAN USE: The Material may not be used: a) in humans; b) in conjunction with human clinical trials; c) in association with human diagnostics.

MATERIAL NOT TRANSFERABLE: You may not transfer the Material to any other person or organization.


Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza warrants its cells only if Clonetics® Media and Reagents are used and the recommended protocols are followed.

Cryopreserved Sertoli Cells are assured to be viable and functional when thawed and maintained properly. Sertoli Cells can become irreversibly contact-inhibited if allowed to reach confluence. To avoid the loss of your cells and forfeiture of your warranty, subculture cells before they reach 80% confluence.

Quality Control

All cells are performance assayed and test negative for HIV-1, Hepatitis B & C, mycoplasma, bacteria, yeast and fungi. Cell viability and morphology are measured after recovery from cryopreservation. Sertoli Cells are ≥ 70% positive for GATA-4 and SOX-9 by flow cytometry. For detailed information concerning QC testing, please refer to the Certificate of Analysis.

When placing an order or for technical 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.