

Human Skeletal Muscle Derived Cells (SkMDC)
Catalog #SK-1111



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Instructions for Use

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Use Restrictions

This product is distributed for research use only. The use of this product is not approved for human or veterinary use. Do not use this product with in vitro diagnostics.

Buyer has no rights to transfer products, components, or materials made using these products, or to use these products for commercial purposes. Commercial purposes include: use of products or their components in manufacturing; use of products or their data components to provide a service, information or data; use of products or their components for therapeutic or diagnostic purposes; resale of products or their components.

Safety

Products contain human source material. Treat as potentially infectious. Handle at Biological Safety Level 2 to minimize exposure to potentially infectious agents. Each donor tissue, prior to cell isolation, is tested for the presence of HIV-1, Hepatitis B and Hepatitis C virus. Testing cannot offer complete assurance that tested virus is absent.

Handling of Frozen Cells

Cells arrive frozen on dry ice. If no dry ice is present in shipping container, contact customer support. To ensure the highest level of cell viability, thaw and initiate culture immediately upon receipt. It is recommended that, if not using immediately, or for continued storage, the frozen cryovial be placed in a -80°C freezer (short term, < 1 day) or liquid nitrogen vapor phase (long term). Cryovials submerged in liquid phase may fill with liquid nitrogen and, upon thawing, explode. **Note:** When handling frozen cells, it is recommended that protective gloves, clothing and eyewear be worn.

Aseptic Technique

The use of aseptic technique is required for all culturing activities. This includes the use of sterile materials and appropriate environmental conditions to ensure sterility when manipulating cultures. Use of reagent aliquotting, rather than repeated use of large volumes (e.g., entire bottle), along with the use of disposable materials/items, is highly recommended. The use of vented culture flasks rather than loosened caps is highly recommended.

Required Reagents

The following reagents are required for optimal culturing of Cook MyoSite SkMDC. These reagents are not included and must be purchased separately. Use of substitute media or reagents may result in poor culture growth and/or performance.

- MyoTonic™ family of growth culture medium products, choose from:
 - MyoTonic™ Growth Medium Kit, **Cook MyoSite Catalog #MK-4444**
 - MyoTonic™ Insulin-Free Growth Medium Kit, **Cook MyoSite Catalog #MK-6633**
 - *MyoTonic™ Serum-Free Growth Medium Kit, **Cook MyoSite Catalog #MK-2288**
 - *MyoTonic™ Insulin-Free Serum-Free Growth Medium Kit, **Cook MyoSite Catalog #MK-6688**

[*FBS must be added prior to use to ensure proper performance]

- MyoTonic™ Differentiation Medium, **Cook MyoSite Catalog #MD-5555**
- Phosphate buffered saline (Mg^{2+}/Ca^{2+} -free) (or equivalent balanced salt solution) - **(not supplied)**
- Passaging enzyme (e.g., trypsin/EDTA) - **(not supplied)**
- CryoTonic™ Cryostorage Medium, **Cook MyoSite Catalog #CR-9999**

Culture Maintenance/Medium Change

1. Cultures should be examined every day and maintained at $\leq 50\%$ confluence (i.e., the amount of surface area occupied by the cells).
2. Culture medium should be changed every other day if passaging does not occur within this time.
3. Prepare aliquots of warm culture medium for each culture medium change.

Thawing Frozen Cells/Culture Establishment

1. Cells from one cryovial (5.0×10^5 cells per vial) should be used to create one T-75 starting culture. Alternatively, cells may be seeded in other culture flasks/wells at a density of 5,000-7,500 cells/cm².
2. Prepare an aliquot of warm (37°C) culture medium prior to thawing. Warm 15 mL of culture medium for a T-75 size flask (0.2 mL/cm² for other flask sizes/wells). **Note:** It is recommended that aliquots of culture medium be prepared and warmed rather than repeatedly warming entire culture medium bottles.
3. Prepare a 3 mL aliquot of culture medium to be used to dilute the thawed cell suspension.
4. Wipe cryovial with 70% ethanol or isopropanol prior to opening. **Note:** Open cryovial one-quarter turn in a sterile environment prior to thawing to relieve potential internal pressure.
5. Submerge bottom $\frac{3}{4}$ of cryovial in a 37°C water bath until contents are visibly thawed. **Note:** Do not submerge cryovial above cap level.
6. Immediately transfer thawed cell suspension to a conical tube and use 3 mL cold culture medium aliquot to rinse cryovial and dilute thawed cell suspension.
7. Centrifuge cell suspension (1,000 xg for five minutes).
8. Remove supernatant from centrifuged tube down to the cell pellet.
9. Resuspend cell pellet thoroughly using a portion of the warmed culture medium and add to the culture flask. Use the remaining warmed culture medium to rinse the tube and add the entire remaining culture medium to the culture flask.
10. Transfer culture flask to 37°C CO₂ humidified incubator.

Subculture (Passaging) Procedure

1. Cultures should be passaged into flasks at a ratio of 1:3 (i.e., one T-75 flask should be passaged into three T-75 flasks, or an equivalent total flask area of 225 cm²). Note: It is not recommended that cultures be passaged if confluence is < 10%. Passaging prior to this confluence may result in decreased population growth.
2. Prepare an aliquot of warm (37°C) culture medium adequate for the new culture flask(s) (0.2 mL/cm²).
3. Warm (37°C) an adequate amount of passaging enzyme (e.g. trypsin/EDTA, 1x), for passaging. Use 3 mL for each T-75 flask to be passaged (adjust volume accordingly for other flask sizes).
4. Prepare an aliquot of culture medium to be used to dilute the cell suspension. Use 9 mL for each T-75 flask (adjust volume to 3x the amount of passage enzyme used for other flask sizes).
5. Remove the culture medium from the flask and immediately replace with phosphate buffered saline. Use 5-10 mL of buffered saline for each T-75 flask (adjust volume accordingly for other flask sizes).
6. Remove the buffered saline and immediately add the appropriate amount of warmed passage enzyme to the flask. Rock the flask to ensure coverage of the entire culture surface with the passage enzyme solution.
7. Examine the flask microscopically to determine when > 90% of the cells have become detached from the surface. **Note:** Flask may be placed at 37°C and/or agitated to facilitate cell removal.
8. Upon confirmation of cell detachment, immediately place ½ of the culture medium aliquot (prepared in step 4) into the culture flask.
9. Remove cell suspension from the culture flask and place into new conical tube. Use the remaining culture medium aliquot to rinse the flask surface and add to the conical tube containing the cell suspension.
10. Centrifuge the cell suspension (1,000 xg for 5 minutes).
11. Remove supernatant from the centrifuged tube down to the cell pellet.
12. Resuspend the cell pellet thoroughly in 3 mL of prepared warm culture medium (prepared in step 2).
13. Distribute this 3 mL cell suspension equally among new flasks (see step 1).
14. Use 3 mL of new warm culture medium to rinse the conical tube and distribute equally among new flasks.
15. Place the remaining warm culture medium into each new flask.
16. Transfer culture flasks to 37°C CO₂ humidified incubator.

Cryopreservation Procedure

1. Follow instructions for Subculture (Passaging) Procedure, steps 3 through 11.
2. Resuspend the cell pellet thoroughly in an appropriate amount of culture medium (use amount to obtain an appropriate concentration for counting method).
3. Determine total viable cell number in cell suspension.
4. Centrifuge cell suspension (1,000 xg for 5 minutes).
5. Resuspend cell pellet thoroughly in a volume of cryopreservation medium to obtain $\geq 5.0 \times 10^5$ viable cells/mL.
6. Transfer aliquot(s) to cryopreservation vial(s).
7. Use appropriate equipment to obtain controlled freezing rate of approximately 1°C/min.
8. Store at -80°C for short time periods (< 1 week) or liquid nitrogen vapor phase for extended storage.

Differentiation Procedure

1. Prepare an aliquot of warm (37°C) differentiation medium adequate for the culture flask(s)/well(s) (0.2 mL/cm²).
2. Aspirate growth culture medium from flask(s)/well(s) and replace with warmed differentiation medium.
3. Differentiation of SkMDC cultures will become visually apparent within 2-4 days following replacement of growth medium with differentiation medium. Differentiation will be marked by the presence of elongated, multi-nucleated myotubes.
4. Differentiation medium should be replaced after 4 days (and every 4 days thereafter). Be careful not to dislodge differentiated myotube structures from the culture surface during medium changes.

Note: Higher cell culture density, $\geq 75\%$ confluence (i.e., the amount of surface area occupied by the cells), is recommended for a more robust differentiation response.

Additional Information

Primary cultures have a finite lifespan and limited number of population doublings in vitro. Cryopreserved cells are tested prior to shipment for contaminating agents. Contamination of cell cultures may affect cell growth, function and behavior. For detailed information concerning quality control testing, specifications and characterization, please refer to Certificate of Analysis provided for each cryopreserved cell lot.

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