Description

Organism: Homo sapiens, human
Tissue: Skin; foreskin
Disease: Normal
Cell Type: Fibroblast
Age: Newborn
Gender: male
Morphology: adherent
Growth Properties: Adherent

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & Storage Instructions

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until ready for use.

Handling Procedure for Frozen Cells

It is not necessary to coat flasks with gelatin prior to plating cells, if tissue culture quality flasks are used. To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells. Cells should be plated at a minimum cell density of 0.8 X 10^4 cells/cm^2.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water.
2. Remove the vial from the water bath as soon as the contents are half way thawed (approximately 90 seconds), and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial’s contents plus 5 mL of complete growth medium to a 15 mL centrifuge tube. Use an additional 1 mL of media to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 mL of complete growth media to bring the total volume to 10 mL.
4. Gently mix and pellet the cells by centrifugation @ 270 xg for 5 minutes.
5. Discard the supernatant and resuspend the cells with 10 mL fresh growth medium (warm) and plate the cells at seed density of 0.8 X 10^4 cells/cm^2.
6. Add more fresh growth medium (warm) to obtain the total volume recommended for the flask.
7. Incubate 37°C in a 5%CO2 in air atmosphere.

Fluid change twice a week or when pH decreases. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

Subculturing Procedure

Procedure:

To insure the highest level of viability, be sure to warm media and Trypsin/EDTA to 37°C before using it on the cells.

Cells should be split when they reach confluency. A split ratio of 1:5 to 1:7 is recommended. Volumes used in this protocol are for 225 cm^2 (T225); proportionally reduce or increase amount of dissociation medium for culture flasks of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 1X PBS (SCRR-2201) solution to remove all traces of serum, which contain trypsin inhibitor.
3. Add 5 mL of Trypsin-EDTA (0.25% (w/v) Trypsin-0.53 mM EDTA solution, ATCC# 30-2101) solution to
add 6.0 to 8.0 mL of complete growth medium and rinse surface of the flask to detach all cells. Gently
Add 10 mL complete growth medium to cell pellet and with 10 mL pipette resuspend the cells gently
Add more complete growth medium to cell suspension as needed to plate cells at approximately
from the U.S.
Remove and discard the supernatant
Transfer all cells into a centrifuge bottle or tube and centrifuge at 270 xg for 5 minutes.
Place flasks in incubator @ 37°C with a 5% CO2 atmosphere. (Standard DMEM formulations contain 3.7
Twice a week or as pH decreases
Biosafety Level: 1

<table>
<thead>
<tr>
<th>Flask/Plate</th>
<th>Growth Area</th>
<th>1xPBS</th>
<th>Trypsin/EDTA</th>
<th>Equal vol. Complete Growth Medium</th>
<th>Growth Medium</th>
</tr>
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<tbody>
<tr>
<td>T225</td>
<td>225</td>
<td>10 ± 0.2</td>
<td>6 ± 0.2</td>
<td>6 ± 0.2</td>
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<tr>
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<td>3 ± 0.1</td>
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<tr>
<td>T25</td>
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<td>3 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>6</td>
</tr>
<tr>
<td>6 well</td>
<td>9.5</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
<td>1 ± 0.1</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 13 in
Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 5th edition, published by Alan R.

Subcultivation Ratio: A subcultivation ratio of 1:5 to 1:7 is recommended
Medium Renewal: Twice a week or as pH decreases

Cryopreservation Medium
Complete growth medium supplemented with an additional 40% FBS and 10% (v/v) DMSO
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments
The growth of these cells should be arrested before being used as a feeder layer. ATCC has successfully
irradiated (SCRC-1041.1) and treated the cells with Mitomycin C (SCRC-1041.2) for use as a feeder layer. If
the HFFs are being used as a feeder layer for ES cells, it is not recommended to use them past passage no.
50 (P50). It is recommended that the feeder cells be plated 24 hours before use at 5X10⁴ cells/cm² in order to
obtain a supportive monolayer for stem cell growth.

References
References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the
current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S.
Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes
for Health.

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