Adherent

Gently mix and pellet the cells by centrifugation @ 270 xg for 5 minutes. Check all containers for leakage or breakage. Remove and discard culture medium. Incubate 37°C.

Add 5 mL of Trypsin-EDTA (0.25% (w/v) Trypsin-0.53 mM EDTA solution, ATCC# 30-2101) solution to Briefly rinse the cell layer with 1X PBS (SCRR-2201) solution to remove all traces of serum, which 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. 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. Gently mix and pellet the cells by centrifugation @ 270 xg for 5 minutes. Discard the supernatant and resuspend the cells with 10 mL fresh growth medium (warm) and plate the cells at seed density of 0.8 X10^4 cells/cm^2. Add more fresh growth medium (warm) to obtain the total volume recommended for the flask. 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 7.8).

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HFF-1 (ATCC® SCRC-1041™)
Add 6.0 to 8.0 mL of complete growth medium and rinse surface of the flask to detach all cells. Gently pipetting up and down will break cell clumps.
4. Add 10 mL complete growth medium to cell pellet and with 10 mL pipette resuspend the cells gently (create a single-cell suspension).
5. Add more complete growth medium to cell suspension as needed to plate cells at approximately 5x10^5/T225 flask.
6. Place flasks in incubator @ 37°C with a 5% CO2 atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO2 in air atmosphere)
7. Twice a week or as pH decreases
8. Add more complete growth medium to cell suspension as needed to plate cells at approximately 5x10^5/T225 flask.
9. Place flasks in incubator @ 37°C with a 5% CO2 in air atmosphere.

<table>
<thead>
<tr>
<th>Flask/Plate</th>
<th>Growth Area (cm²)</th>
<th>1xPBS (mL)</th>
<th>Trypsin/EDTA (mL)</th>
<th>Equal vol. Complete Growth Medium (mL)</th>
<th>Growth Medium (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T225</td>
<td>225</td>
<td>10 ± 0.2</td>
<td>6 ± 0.2</td>
<td>6 ± 0.2</td>
<td>30</td>
</tr>
<tr>
<td>75</td>
<td>75</td>
<td>5 ± 0.1</td>
<td>3 ± 0.1</td>
<td>3 ± 0.1</td>
<td>12</td>
</tr>
<tr>
<td>T25</td>
<td>25</td>
<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>


Subcultivation Ratio: A subcultivation ratio of 1:5 to 1:7 is recommended

Medium Renewal: Twice a week or as pH decreases

Cryopreservation Medium

Dulbecco’s Modified Eagle’s Medium 30-2002, 7% FBS, 10% (v/v) DMSO. Lots produced prior to May 2019 may have used a different cryopreservation medium (complete growth medium supplemented with an additional 40% FBS and 10% (v/v) DMSO), contact Technical Support for further details.

Comments

The growth of these cells should be arrested before being used as a feeder layer. ATCC has successfully irradiated (ATCC SCRC-1041.1) and treated the cells with Mitomycin C (ATCC 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 PDL 45. It is recommended that the feeder cells be plated 24 hours before use at 5 x 10^4 cells/cm^2 in order to obtain a supportive monolayer for stem cell growth.

References

Please read this FIRST

Storage Temp.
Liquid nitrogen
vapor phase

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Dulbecco’s Modified Eagle’s Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium:

- 15% fetal bovine serum
- 2 mM L-glutamine
- 100 units/mL penicillin
- 100 μg/mL streptomycin

This medium is formulated for use with a 5% CO2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO2 in air atmosphere is then recommended).

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HFF-1 (ATCC® SCRC-1041™)

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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Complete Growth Medium

The base medium for this cell line is ATCC-formulated Dulbecco’s Modified Eagle’s Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 15%

This medium is formulated for use with a 5% CO2 in air atmosphere. (Standard DMEM formulations contain 3.7 g/L sodium bicarbonate and a 10% CO2 in air atmosphere is then recommended).

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HFF-1 (ATCC® SCRC-1041™)