ATCC

Product Sheet

HFF-1 (ATCC® SCRC-1041™)

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:

- 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™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: Skin; foreskin
Disease: Normal
Cell Type: Fibroblast
Age: Newborn
Gender: male
Morphology: fibroblast
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 $0.8X10^4$ 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 $0.8\ X10^4$ 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%CO_2 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 37ºC 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

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flask and incubate for 1 minute, gently tapping the flask observe cells under an inverted microscope until cells detach (usually within 1 to 2 minutes).

4. 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.
5. Transfer all cells into a centrifuge bottle or tube and centrifuge at 270 xg for 5 minutes.
6. Remove and discard the supernatant
7. Add 10 mL complete growth medium to cell pellet and with 10 mL pipette resuspend the cells gently (create a single-cell suspension).
8. Add more complete growth medium to cell suspension as needed to plate cells at approximately 5x10^6/T225 flask.
9. Place flasks in incubator @ 37°C with a 5% CO_2 in air atmosphere.

Flask/Plate	Growth Area (cm^2)	1xPBS (mL)	Trypsin/EDTA (mL)	Equal vol. Complete Growth Medium (mL)	Growth Medium (mL)
T225	225	10 ± 0.2	6 ± 0.2	6 ± 0.2	30
75	75	5 ± 0.1	3 ± 0.1	3 ± 0. 1	12
T25	25	3 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	6
6 well	9.5	1 ± 0.1	1 ± 0.1	1 ± 0.1	3

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. Liss, N.Y., 1994.
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 5X10^4 cells/cm^2 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.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the

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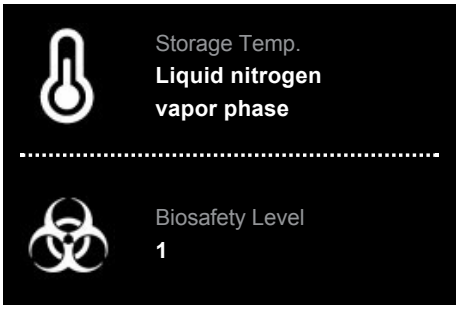

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product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.
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