



Product Sheet

AFT024 IRR (ATCC® SCRC-1007.1™)

Please read this FIRST



Storage Temp.
**liquid nitrogen
vapor phase**



Biosafety Level
2

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: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: AFT024 IRR (ATCC® SCRC-1007.1™)

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: *Mus musculus*, mouse
Tissue: Liver; stroma
Cell Type: Fibroblast SV40 immortalized, SV40 transformed
Age: 14 to 14.5 day gestation embryo
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

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C . Storage at -70°C will result in loss of viability. To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells.

Flasks do not need to be coated before plating MEFs.

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 medium (see below for recipe) to a 15 mL centrifuge tube. Use an additional 1 mL of medium to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 mL of complete medium to bring the total volume to 10 mL.
4. Gently mix and pellet the cells by centrifugation @ $270 \times g$ for 5 minutes.
5. Discard the supernatant, resuspend the cells in fresh growth medium (warm), and transfer to the appropriate size flask.
6. Incubate 37°C in a 5% CO_2 in air atmosphere.
7. 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).

Cells should be plated 24 hours before use as a feeder layer for ES cells and kept for no more than 7 days.

Handling Procedure for Flask Cultures

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

Flasks do not need to be coated before plating MEFs.


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 medium (see below for recipe) to a 15 mL centrifuge tube. Use an additional 1 mL of medium to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 mL of complete medium to bring the total volume to 10 mL.




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4. Gently mix and pellet the cells by centrifugation @ 270 xg for 5 minutes.
5. Discard the supernatant, resuspend the cells in fresh growth medium (warm), and transfer to the appropriate size flask.
6. Incubate 37°C in a 5% CO₂ in air atmosphere.
7. 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).

Cells should be plated 24 hours before use as a feeder layer for ES cells and kept for no more than 7 days.



Subculturing Procedure

Medium Renewal: twice a week or when pH decreases



Comments

These cells have been growth-arrested by irradiation with 12,000 rads. The cells will begin to deteriorate 2 weeks after plating and may no longer support the growth of cells. We recommend that you do not keep the cells in culture for longer than 2 weeks.

It is recommended that the feeder cells be plated 24 hours before use at 5 to 6 X 10⁶ cells/T75 in order to obtain a 100% confluent monolayer for stem cells growth. Once the feeder cells have attached, the culture medium can be changed to accommodate the cells to be supported.



References

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



Biosafety Level: 2

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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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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