



Product Sheet

TH-1, Subline B1 (ATCC® CCL-50™)

Please read this **FIRST**



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 Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 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: TH-1, Subline B1 (ATCC® CCL-50™)

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: *Terrapene carolina*, turtle, box

Morphology: epithelial

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

HANDLING PROCEDURE FOR FROZEN CELLS

- Initiate culture as soon as possible upon receipt.

- Thaw by rapid agitation in 23°C water bath. Thawing should be rapid (within 40-60 seconds). As soon as the ice is melted, remove the ampule from the water bath and immerse in 70% ethanol at room temperature. All of the operations from this point on should be carried out under strict aseptic conditions.

- Transfer the cell suspension and dilute it with the recommended culture medium in a culture flask (see specific batch information above for dilution ratio); incubate at 23°C in a closed system without CO₂ in air atmosphere. Since it is important to avoid excessive alkalinity of the medium during recovery of the cells, it is suggested that the culture medium be placed into the culture flask, tube, etc. and the pH be adjusted, as necessary, prior to the addition of the ampule contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing CO₂ will be required.

- It is not necessary to remove the freezing additive. However, if desired, the culture medium may be changed to remove the protective freezing additive (dimethylsulfoxide) 24 hours after thawing. If it is desired that the freezing additive be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information above.

FLUID RENEWAL

Every 10-14 days.

SUBCULTURE PROCEDURE

Remove medium, add fresh trypsin (0.25%) solution for 2-3 minutes. Remove and place culture at room temperature for 5-10 minutes until cells detach. Add fresh culture medium, aspirate and dispense into new flasks.

Subcultivation ratio: 1:2.

Handling Procedure for Flask Cultures

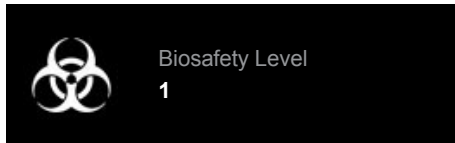
The flask was seeded with cells (see specific batch information above for



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concentration), grown and completely filled with medium to prevent loss of cells in transit. Remove all of the medium (which can be saved and used as fresh medium) except for a sufficient volume (5-10 ml) to cover the floor of the flask. Incubate at 23°C in a closed system without a CO₂ in air atmosphere. Sometimes in transit the cultures are handled roughly and most of the cells become detached and float in the culture medium. If this has occurred remove the entire contents of the flask and centrifuge at 300 x g for 15 minutes. Draw off the excess supernatant medium, resuspend the cells in 10 ml of the culture medium and plant the entire cell suspension in a single flask of suitable size (about 25 sq. cm.).



Subculturing Procedure

Subcultivation Ratio: A subcultivation ratio of 1:2 is recommended

Medium Renewal: Every 10 to 14 days

Remove medium, add fresh 0.25% trypsin for 2 to 3 minutes, remove trypsin and allow the culture to stand for 5 to 10 minutes at room temperature. Add fresh medium, aspirate and dispense into new flasks.



Comments

Growth is inhibited at alkaline pH. Medium should be adjusted to neutrality after plating.



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

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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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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