



Product Sheet

# MITC-STO (ATCC 56-X) (ATCC® 56-X.2™)

Please read this **FIRST**



Storage Temp.  
**liquid nitrogen  
vapor phase**

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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 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: MITC-STO (ATCC 56-X) (ATCC® 56-X.2™)

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

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Or contact your local distributor

## Description

**Organism:** *Mus musculus*, mouse

**Tissue:** embryo

**Cell Type:** fibroblast

**Age:** 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  $-130^{\circ}\text{C}$ , preferably in liquid nitrogen vapor, until ready for use.

## Handling Procedure for Frozen Cells

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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

$-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

**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.

1. Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, 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 contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately  $125 \times g$  for 5 to 7 minutes.
3. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a culture flask. 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).
4. Incubate the culture at  $37^{\circ}\text{C}$  in a suitable incubator.

## Handling Procedure for Flask Cultures

### Use of Feeder Cells:

1. Select an appropriate culture vessel for co-cultivation, as feeder layer cells cannot be easily transferred by enzymatic dissociation or scraping.
2. Dilute Mitomycin C-treated cells to a concentration such that the culture will be 30% confluent if used for sustaining the growth of hybridoma cells or 100% confluent to keep stem cells undifferentiated. Consider the surface area and volume of the culture vessel.
3. Although feeder cells may be added to the cultures at any time, seeding the feeder layer 24 hours before use is recommended. Before adding primary cells, examine the feeder layer cultures to ensure proper confluence.



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- The fibroblastic feeder layer will deteriorate with continued incubation (2-3 weeks). Add feeder cells as necessary.

### Comments

These cells are generated from mouse embryonic fibroblasts, STO cell line (ATCC CRL-1503), by a treatment of Mitomycin C.

These cells are provided to be used as feeder cells to support the growth of other cells. They have been treated with Mitomycin C and will not replicate. The cells will begin to deteriorate in 2 to 3 weeks after plating. Once the feeder cells have attached, the culture medium can be changed to accommodate the cells to be supported. Such populations are employed for maintenance of embryonal stem cells such as ES-D3 (ATCC CRL-1934) or teratocarcinoma stem cells (see ATCC CRL-1535 and ATCC CRL-1566) in the undifferentiated state.

It is recommended that the feeder cells be plated 24 hours before use at  $6 \times 10^6$ /T-75 or  $2 \times 10^6$ /T-25 in order to obtain a 100% confluent monolayer for stem cells growth.

Feeder layers are also employed to enhance the growth at low density populations of many hybridomas, colon carcinoma cell line (ATCC CCL-247) and squamous cell carcinoma cell lines (ATCC CRL-1624 and ATCC CRL-1629). This action is due partly to conditioning of the substrate and the medium. A feeder layer confluence of 30 % is adequate and can be obtained by plating  $2 \times 10^6$ /T-75.

### References

References and other information relating to this product are available online at [www.atcc.org](http://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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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](http://www.atcc.org)

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).  
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