ATCC

Product Sheet

# *Mouse stem cell line with a disrupted lck gene. Mus musculus* (CRL-2542)

**Please read this FIRST**

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.

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

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**U.S. Patent Number:**
5,625,122

## Technical Information

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## Product Description

**Designation:** Mouse stem cell line with a disrupted lck gene. Mus musculus
**Organism:** *Mus musculus*
, mouse
**Strain:** 129/Sv+c/+p
**Cell Type:** embryonic stem cell
**Age:** embryo
**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**

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.
**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°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.
3. Transfer the vial contents to an appropriate size vessel. 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

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medium to reach its normal pH (7.0 to 7.6).

## Handling Procedure for Flask Cultures

**Handling Procedure for Flask Cultures (Monolayer)**

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% $CO_2$ in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 $cm^2$ flask. Incubate at 37°C in a 5% $CO_2$ in air atmosphere until cells are ready to be subcultured.

## Subculturing Procedure

**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended
**Medium Renewal:** 3 times per week
Remove medium and rinse the monolayer with fresh 0.25% trypsin, 0.03% EDTA solution. Remove the trypsin and incubate at 37°C until the cells detach (approximately 10 minutes). Add fresh medium, aspirate and dispense onto fresh feeder layer cultures.

## Cryopreservation Medium

**Cryoprotectant Medium**

Complete culture medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

## Comments

The replacement-type vector (PmlckBSNeo2.3) was introduced into D3 embryonic stem (ES) cells by electroporation to disrupt the lck locus.
Two cell lines, 59B5 (ATCC-CRL-11115) and 56B3 (ATCC-CRL-11117), were generated that were deficient for the lck gene.
These cell lines were used to produce mutant mice with germ line transmission for lck disruption.
Heterozygous mice were inbred to obtain mice homozygous for the disrupted lck gene.
A culture submitted to the ATCC as CRL-11117 in September of 1992 was found to be contaminated with mycoplasma. Progeny were cured by a 21-day treatment with BM Cycline.
The cured cell line is available as CRL-2542. The original patent deposit is available as CRL-11117.
The line should be grown on feeder layers of mitomycin C treated (0.01 mg/ml for 90 minutes) primary mouse embryonic fibroblasts or STO cells (see ATCC CRL-1503 or ATCC 56-X.2, MITC-STO cells).

## Propagation

**Complete Growth Medium**

Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose and supplemented with 0.1 mM 2-mercaptoethanol and 10mM HEPES, 85%; fetal bovine serum, 15%

## 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

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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**

Patent Deposits not produced or characterized by ATCC are warranted for viability only. If you believe the culture you have received is nonviable, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

**Disclaimers**

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**Disclosure**

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

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