



# Shox2

CRL-3256™

## Description

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

**Cell Type:** embryonic stem cell, nodal cardiac myocyte-derived

**Tissue:** Heart; Sinoatrial node

**Age:** embryo

**Gender:** Male

**Morphology:** epithelial-like

**Growth properties:** Adherent

**Volume:** 1.0 mL

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** Vapor phase of liquid nitrogen

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## Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

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## BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local

or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

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## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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## Growth Conditions

**Temperature:** 37°C

**Atmosphere:** 95% Air, 5% CO<sub>2</sub>

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## Handling Procedures

### Unpacking and 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.

**Complete medium:** The base medium for this cell line is Mouse ES Cell Basal Medium,

Catalog No. SCRR-2011. 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 15%

**Handling Procedure:** 1 amp --> 1 T-75

Thaw ampoule in 37°C water bath for approximately 2 minutes. Transfer thawed cell suspension to a 15.0 mL centrifuge tube containing 9mL complete medium. Mix suspension by gentle inversion. Remove 0.5-1.0 mL for cell count. Centrifuge remaining suspension in the 15 mL centrifuge tube at 175-195 x g (1000 rpm in an IEC HN SII centrifuge or equivalent) for 5 minutes, RT. Discard supernatant and gently resuspended pellet in 5ml fresh complete medium. Transfer 5 ml re-suspended cells into 1 T-75 flask containing 10 ml fresh medium. Place the cells in a 5% CO<sub>2</sub> incubator @ 37°C

**Subculturing procedure:**

Volumes used in this protocol are for 75 cm<sup>2</sup> flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium. Briefly rinse the cell layer with Ca<sup>++</sup>/Mg<sup>++</sup> free Dulbecco's phosphate-buffered saline (D-PBS) (ATCC 30-2200) or 0.25% (w/v) Trypsin - 0.53 mM EDTA (ATCC 30-2101) solution to remove all traces of serum which contains trypsin inhibitor.
2. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).

**Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

1. Add 2.0 to 3.0 ml of complete growth medium and aspirate cells by gently pipetting.
2. Transfer cell suspension to a centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.
3. Discard supernatant. Resuspend the cell pellet in fresh growth medium.
4. Add appropriate aliquots of the cell suspension to new culture vessels.
5. Incubate cultures at 37°C.

**Subcultivation Ratio:** 1:3 to 1:6 is recommended.

**Medium Renewal:** 2 to 3 times a week

**Culture maintenance:** Cultures are grown @ 37°C in a 95% air, 5% CO<sub>2</sub> environment. Medium change every 2-4 days.

**Reagents for cryopreservation:** Fetal Bovine Serum supplemented with 5% (v/v) DMSO (ATCC 4-X)

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## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Shox2 (ATCC CRL-3256)

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## References

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

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