



# B9013HCB

CRL-3474™

Product Sheet

## Description

B9013HCB is a cell line exhibiting spindle-like morphology that was isolated from the skin of an adult female mouse with melanoma. This cell line is a mouse model of melanoma and can be used for building preclinical models for therapeutic studies, including immunotherapies.

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

**Tissue:** Skin

**Age:** adult

**Gender:** Female

**Morphology:** Spindle-like

**Growth properties:** Adherent

**Disease:** Melanoma

**Cells per vial:** Approximately 2.0 to 3.0 x 10<sup>6</sup>

**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 80% MCDB-153 (Sigma Aldrich catalog # F8105-500ML) 20% Leibowitz -15 Medium (ATCC 30-2008). To make the complete medium add the following components:

- 2% fetal bovine serum (FBS; ATCC 30-2020)
- 2 mM L-Glutamine (ATCC 30-2214)
- 1.68 mM Calcium Chloride (1M stock; VWR catalog #E506-100ML or equivalent.)
- 5 µg/mL Gibco™ Insulin, human recombinant, zinc solution (4mg/mL stock; ThermoFisher catalog # 12585-014)

**Handling Procedure:**

To ensure 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.

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 a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x *g* for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 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 complete 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). pH (7.0 to 7.6).

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO<sub>2</sub> in air atmosphere is recommended if using the medium described on this product sheet.

**Subculturing procedure:**

Volumes used in this protocol are for 75 cm<sup>2</sup> flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.05% Trypsin – 0.02% EDTA to remove all traces of serum that contains trypsin inhibitor.
3. 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.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Removal of Dissociation Agent: Centrifuge cells 150 to 400 x g; 8 to 12 minutes. Remove supernatant and resuspend the pellet in culture medium. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 1.5 x 10<sup>4</sup> and 3.0 x 10<sup>4</sup> viable cells/cm<sup>2</sup>.
6. Incubate cultures at 37°C.

**Interval:** Maintain cultures at a cell concentration between 1.0 X 10<sup>4</sup> and 3.0 X 10<sup>4</sup> cell/cm<sup>2</sup>.

**Subcultivation Ratio:** A Subcultivation ratio of 1:3 to 1:8 is recommended

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

**Reagents for cryopreservation:** MCDB-153 + 20% L-15 + 2% FBS + 2 mM L-Glutamine + 1.68 mM Calcium Chloride + 5 µg/mL human recombinant insulin + 5% DMSO

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**Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: B9013HCB (ATCC CRL-3474)

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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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