



WEHI-3B cell line

Catalog Number: PC-032 (For Research Use Only)

Product Overview

The WEHI-3B cell line is a murine myelomonocytic leukemia suspension cell line widely used as a source of interleukin-3 (IL-3). Conditioned medium collected from WEHI-3B cultures provides a cost-effective alternative to recombinant IL-3 for maintaining IL-3-dependent cell lines such as Ba/F3.

Materials provided

One vial of 2 x 10⁶ cells in Freezing Media. **IMPORTANT:** store the frozen cells in liquid nitrogen until you are ready to thaw and propagate them.

Handling cells upon arrival

It is strongly recommended that you propagate the cells by following instructions as soon as possible upon arrival.

Required Cell Culture Media

- **Complete Growth Media**
In 450mL of RPMI-1640, add 50mL FBS (10% final) and 5mL Penicillin/Streptomycin (1% final).
- **Freezing Media**
Add 10% DMSO (final) to 70% Complete Growth Media and 20% FBS. Make fresh each time.

Materials required but not provided

(Can be substituted with a comparable third-party product)

- RPMI-1640 Medium -- *Hyclone P/N SH30027.01*
- Fetal Bovine Serum (FBS) -- *Fisherbrand P/N 03-600-511*
- Penicillin/Streptomycin -- *Hyclone P/N SV30010*
- Trypsin – *Hyclone P/N SH30236.02*
- Phosphate-buffered saline (PBS) -- *Cellgro P/N 21-040-CV*

- DMSO -- *Sigma P/N D8418*

Initial Culture Procedure

1. Quickly thaw cells in a 37°C water bath with careful agitation. Remove from the bath as soon as the vial is thawed, and decontaminate by dipping in or spraying with 70% ethanol.
2. Transfer cells to a 100mm² dish (or T25cm² flask) containing 10-12ml of **Complete Growth Media**.
3. Gently rock the flask to ensure the cells are mixed well in the media. DO NOT PIPET.
4. Place the flask with cells in a humidified incubator at 37°C with 5% CO₂.

Subculture Procedure

1. Subculture/passage cells when the density reaches 90-100% confluency.
2. Maintain cell density between 2×10⁵ and 2×10⁶ viable cells/ml.

Preparing frozen stocks

This procedure is designed for 100mm² dish or T75cm² flask. Scale volumes accordingly to other vessels.

1. When cells reach 90-100% confluency, freeze down cells.
2. Detach cells according to “Subculture Procedure.”
3. Transfer cells to a 15ml conical centrifuge tube and centrifuge at 125 x g for 5 to 7 minutes to collect the cells into a pellet.
4. Carefully aspirate the media and add 1mL of freezing media and gently resuspend by pipetting up and down.
5. Transfer 1mL of cells into a cryogenic vial.
6. Place cryogenic vial in a freezing container (*Nalgene # 5100-0001*) and store at -80°C freezer overnight.
7. Transfer cells to liquid nitrogen for long term storage.