



FLT3 D835N Stably Expressing Ba/F3 Cell Line

Cat# SL-0116 (For Research Use Only)

Introduction

The FMS-like tyrosine kinase 3 (FLT3) also known as cluster of differentiation antigen 135 (CD135) or fetal liver kinase-2 (Flk2) is a class III receptor tyrosine kinase (RTK) that plays a key role in controlling survival, proliferation, and differentiation of hematopoietic cells and it widely expresses in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Mutations in FLT3 (both internal tandem duplications (ITD) and point mutations) are among the most common somatic mutations in AML patients and are associated with poor prognosis and outcomes in those patients. Studies showed that many cancer patients with point mutations in the tyrosine kinase domain of FLT3, typically at the activation loop (AL) residue D835 would acquire clinical resistance to FLT3 inhibitors like sorafenib, quizartinib, ponatinib, and crenolanib.

Signosis has generated a series of FLT3 mutants including FLT3 D835V stably expressing in Ba/F3 cell line that can be used for studying the molecular mechanism of resistance in these mutant cell lines or screen for a new compound.

Materials provided

One vial of $2-3 \times 10^6$ cells, in Freezing Media. **IMPORTANT:** store the vial in liquid nitrogen until you are ready to thaw and propagate them.

Handling cells upon arrival

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

IMPORTANT: Please thaw and culture the cells upon arrival**. Also, an adequate number of frozen stocks must be made from early passages as cells will undergo genotypic changes. Genetic instability in transfected cells will result in a decreased responsiveness over time in normal cell culture conditions.

Required Cell Culture Media

- **Complete Growth Media**
In 450mL of RPMI medium, add 50mL FBS (10% final), and 5mL Penicillin/Streptomycin (1% final).

Note: add Murine IL-3 at final concentration of 10ng/ml.

- **1x Freezing Media**
Add 10% DMSO (final) to Complete Growth Media and sterile filter. Make fresh each time.

Materials required but not provided
(can be substituted with a comparable third-party product)

- RPMI-1640 Medium (RPMI) -- *Hyclone P/N SH30027.01*
- Fetal Bovine Serum (FBS) -- *Fisherbrand P/N 03-600-511*
- Penicillin/Streptomycin -- *Hyclone P/N SV30010*
- DMSO -- *Sigma P/N D8418*
- Murine IL-3 -- *PeproTech P/N 213-13*

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.
Transfer cells to a 100mm² dish (or T-25cm² flask) containing 8-12ml of **Complete Growth Media with Murine IL-3 at final concentration of 10ng/ml.**
2. Gently rock the plate to ensure the cells are mixed well in the media. DO NOT PIPET.
3. Place the plate with cells in a humidified incubator at 37°C with 5% CO₂.
4. After this incubation time (wait at least 6 hours to overnight), **replace media** with fresh **Complete Growth Media.**

Subculture Procedure

1. Subculture/passage cells when density reaches $0.8-1 \times 10^6$ /ml
2. Passage cells every 3 days by inoculating 5×10^5 or in 1:3 to 1:5 ratio with warm **Complete Growth Media**

NOTE: Stable cell lines may exhibit a slower proliferation rate compared to parental cells. Do not seed cells at suboptimal density as this may hinder cell growth and division.

Preparing frozen stocks

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

1. When cells reach 1×10^6 /ml, freeze down cells.
2. Centrifuge culture at 1000 RPM for 5 minutes to collect the cells into a pellet.
3. Carefully aspirate the media. Resuspend cells at a density of 3×10^6 cells/ml in freshly prepared 1X freezing media and gently resuspend by pipetting up and down.
4. Aliquot 1ml cells into a cryogenic vial.
5. Place the cryogenic vial in a freezing container (Nalgene # 5100-0001) and store it at -80°C freezer overnight.
6. Transfer cells to liquid nitrogen for long-term storage.

Other related stable cell lines

- FLT3 D835F Stably expressing Ba/F3 Cell Line (SL-0117)
- FLT3 D835H Stably expressing Ba/F3 Cell Line (SL-0118)
- FLT3 Stably expressing Ba/F3 Cell Line (SL-0119)
- FLT3 D835V Stably expressing Ba/F3 Cell Line (SL-0114)
- FLT3 D835E Stably expressing Ba/F3 Cell Line (SL-0115)
- FLT3 D835N Stably expressing Ba/F3 Cell Line (SL-0116)

See here for a complete list of stable cell lines:
<https://www.signosisinc.com/category/cell-based-assays>

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