Ba/F3 FLT3-ITD-A848P Cell Line

Cat. No: KC-1196 Version 18033102

Ⅰ. Cell Line Information

Catalog number	KC-1196
Cell line name:	Ba/F3 FLT3-ITD-A848P Cell Line
Gene ID/Accession #:	NM_004119.2
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous FLT3 bearing ITD duplicate and A848P amino acid double mutations
Quantity:	One vial of frozen cells ($5X10^6$ per vial)
Stability:	Stable in culture over a minimum of 10 passages
Application:	Drug screening and biological assays
Freeze medium:	70% RMPI-1640 + 20% FBS + 10% DMSO
Propagation medium:	RPMI 1640 + 10% FBS
Selection marker:	Puromycin
Morphology:	Mostly single, round (some polymorph) cells in suspension
Subculture:	Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10^5 cells/ml
Incubation:	37 ℃ with 5% CO_2
Storage:	Frozen in liquid nitrogen with 70% medium, 20% FBS and 10% DMSO
Doubling time:	Approximately 20 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

Ⅱ. Background

Fms like tyrosine Kinase 3 (FLT3), also named as CD135, FLK2, is receptor kinase receptor, expressed on the surface of many hematopoietic progenitor cells. Overactivation of FLT3 due to mutation and overexpression can lead to

the development and poor prognosis of Leukemia, the identification of FLT3 as a driver gene has led to the repaid development of anti-AML therapeutics, including Sunitinib, Sorafenib and Quizartinib (AC220).
Ba/F3 cell, a murine interleukin-3 dependent pro-B cell line, is a popular system for exploring both kinases and their inhibitors, because some protein kinases can render the Ba/F3 cells to be depended on the activation of the kinases instead of IL-3 supplement, while their inhibitors can antagonize the kinase-dependent growth effects.

III. Cell Line Generation

Ba/F3 FLT3-ITD-A848P cell Line was generated using retrovirus vector expressing human FLT3-ITD-A848P sequence.

IV. Characterization using FACS

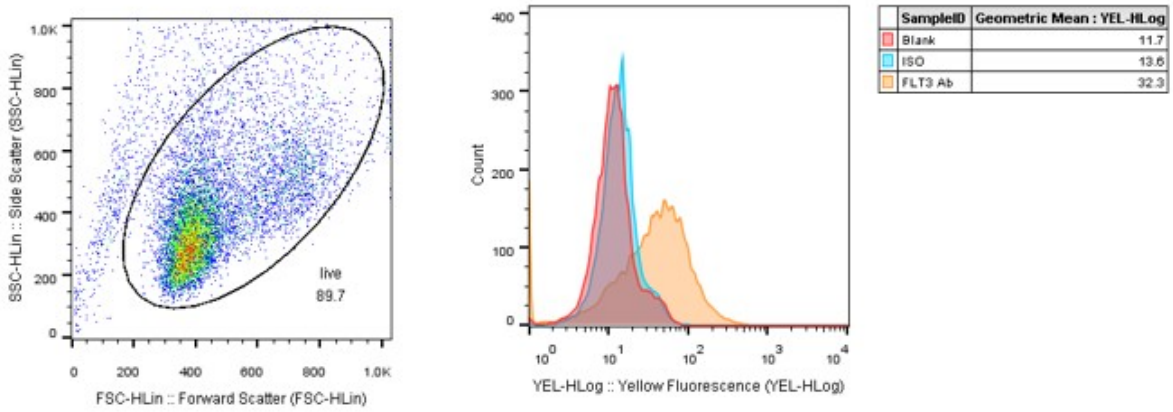
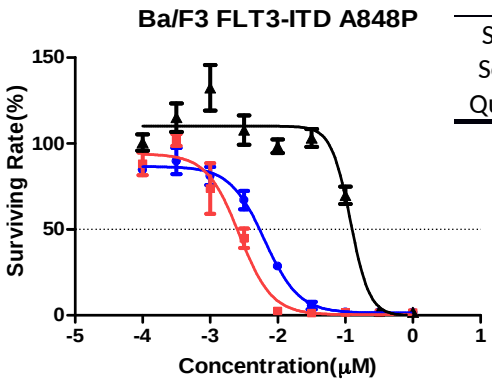


Figure: Characterization of FLT3 and its mutants overexpressing in Ba/F3 stable clones using FACS.

V. Application

a. Cell-based kinase inhibition screen
b. Cell viability assay
c. In vivo efficacy study

Example: kinase inhibitors screening



Compound	IC50 (nM)
Sunitinib	5.37
Sorafenib	125.56
Quizartinib	2.51

IC50 of FLT3 inhibitor

1. Harvest and seed the Ba/F3 cells expressing FLT3 mutant in 96-well plate (3000 cells/90ul medium).
2. Next day, add 10ul 10X serially diluted compound solution each well and incubate the plates for another 72 hours.
3. Add 100ul Cell Titer-Glo each well, mixed and readout using Envision.
4. Plot the dose-responsive curve and fit the IC50 (the centration of 50% inhibition of DMSO vehicle treated clones) using GraphPad Prism software (Version 5).

VI. Cell Resuscitation

1. Prewarm culture medium (RPMI-1640 supplemented with 10% FBS) in a 37℃ water bath.
2. Thaw the frozen vial in a 37℃ water bath for 1-2 minutes.
3. Transfer the vial into biosafety cabinet, and wipe the surface with 70% ethanol.
4. Unscrew the top of the vial and transfer the cell suspension gently into a sterile centrifuge tube containing 9.0 mL complete culture medium.
5. Spin at ~ 125 x g for 5~7 minutes at room temperature, and discard the supernatant without disturbing the pellet.
6. Resuspend cell pellet with the appropriate volume of complete medium and transfer the cell suspension into a T25 culture flask.
7. Incubate the flask at 37℃, 5% CO_2 incubator.
8. Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10^5 cells/ml.

VII. Cell Freezing

1. Prepare the freezing medium (70% RPMI-1640 + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.
3. Transfer cells to a sterile, conical centrifuge tube, and count the cells.
4. Centrifuge the cells at 250 x g for 5 minutes at room temperature and carefully aspirate off the medium.
5. Resuspend the cells at a density of at least 3 x10^6 cells/ml in chilled freezing medium.
6. Aliquot 1 ml of the cell suspension into each cryovial.
7. Freeze cells in the CoolCell freezing container overnight in a −80◦C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

VIII. References

1. Quentmeier, H., Reinhardt, J., Zaborski, M. & Drexler, H. G. FLT3 mutations in acute myeloid leukemia cell lines. Leukemia 17, 120–124 (2003).
2. Smith, C. C. et al. Validation of ITD mutations in FLT3 as a therapeutic target in human acute myeloid leukaemia. Nature 485, 260–263 (2012).
3. Moore, A. S. et al. Selective FLT3 inhibition of FLT3-ITD+ acute myeloid leukaemia resulting in secondary D835Y mutation: a model for emerging clinical resistance patterns. Leukemia 26, 1462–1470 (2012).

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