Ba/F3 EGFR Del19/T790M Cell Line

Cat. No: KC-0113 Version 17081902

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Ⅰ. Cell Line Information

Catalog number	KC-0113
Cell line name:	Ba/F3 EGFR Del19/T790M Cell Line
Gene ID/Accession #:	NM_005228.3
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous EGFR gene with exon19 E746_A750 deletion and T790M 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

EGFR, the Epidermal growth factor receptor, is a cell-surface receptor tyrosine kinase, and activated by binding of

its specific ligand, such as epidermal growth factor (EGF) and transforming growth factor alpha (TGF-alpha), EGFR overexpression or overactivity have associated with a number of cancers, including the lung cancer and colon cancer. The identification of EGFR as a driver gene has led to the development of anticancer therapeutics agents, including Gefitinib, Erlotinib, Afatinib, Osimertinib (AZD9291) and cetuximab.

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 EGFR Del19/T790M cell Line was generated using retrovirus vector expressing human EGFR sequence with exon19 E746_A750 deletion and T790M double mutations.

IV． Characterization using FACS

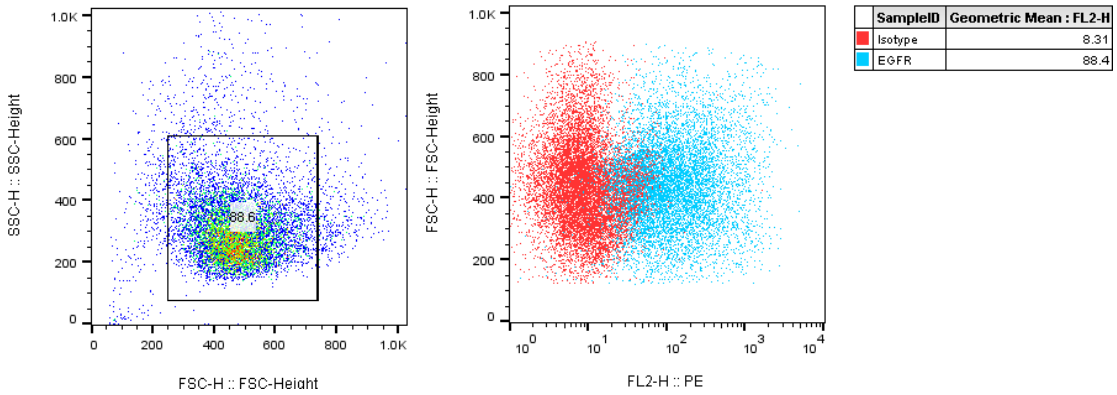
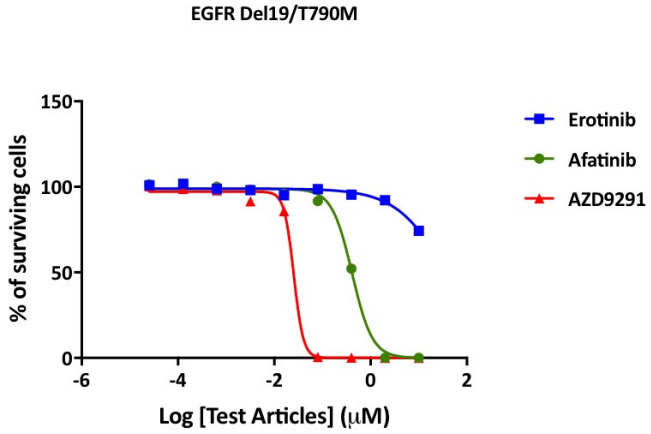


Figure: Characterization of EGFR and its mutants overexpressing in Ba/F3 stable clones using FACS.

V． Application

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

Example: kinase inhibitors screening



IC50 of EGFR inhibitors

Compound	IC50 (uM)
Erlotinib	>10
Afatinib	0.414
AZD9291	0.0254

1. Harvest and seed the Ba/F3 cells expressing EGFR 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℃ freezer.

8. Transfer vials to liquid nitrogen for long-term storage.

VIII． References

1. Kobayashi, S. et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 352, 786–792 (2005).

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