

Ba/F3 EML4-ALK-F1174L Cell Line

Cat. No: KC-0103

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Ba/F3 EML4-ALK-F1174L Cell Line.....	1
I . Cell Line Information.....	1
II . Background.....	1
III . Cell Line Generation.....	2
IV . Characterization using Western Blot.....	2
V . Application.....	2
VI . Cell Resuscitation.....	3
VII . Cell Freezing.....	3
VIII . References.....	3

I . Cell Line Information

Catalog number	KC-0103
Cell line name:	Ba/F3 EML4-ALK-F1174L Cell Line
Gene ID/Accession #:	
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous EML4-ALK fusion gene with F1174L point mutation in the ALK part.
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% RPMI-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 °C 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

EML4-ALK, echinoderm microtubule associated protein like 4 - anaplastic lymphoma kinase, is an abnormal

protein with transforming activity found in some primary lung malignant tumor due to the fusion of abnormal configuration of DNA wherein the echinoderm microtubule-associated protein-like 4 (EML4) gene is fused to the anaplastic lymphoma kinase (ALK) gene; EML4-ALK and its mutants can promote and maintain the malignant behavior of the cancer cells. The identification of ALK as a driver gene has led to the rapid development of anticancer therapeutics agents, including crizotinib, ceritinib and Brigatinib.

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 EML4-ALK cell Line was generated using retrovirus vector expressing human EML4-ALK sequence.

IV . Characterization using Western Blot

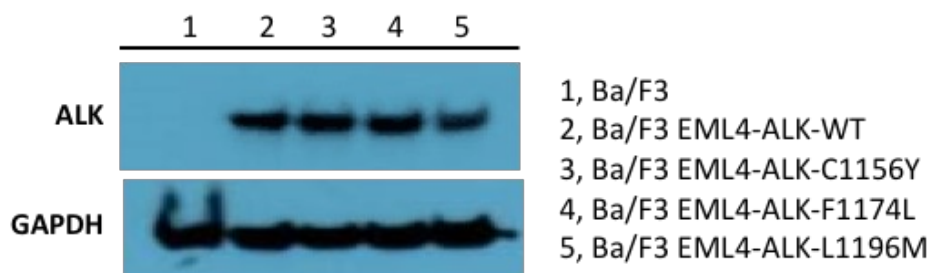
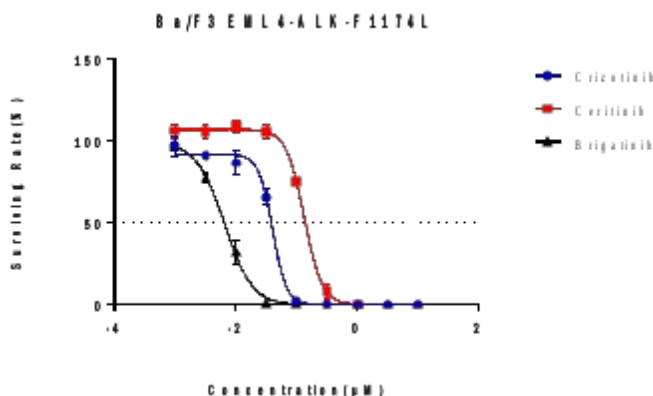


Figure: Characterization of ALK and its mutants overexpressing in Ba/F3 stable clones using Western Blot.

V . Application

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

Example: kinase inhibitors screening



IC50 of ALK inhibitor

Compound	IC50 (nM)
Crizotinib	38.45
Ceritinib	139.52
Brigatinib	6.43

1. Harvest and seed the Ba/F3 cells expressing EML4-ALK 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 concentration 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°C water bath.
2. Thaw the frozen vial in a 37°C 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°C, 5% CO₂ incubator.
8. Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10⁵ 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 x 10⁶ 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. Koivunen, J. P. et al. EML4-ALK Fusion Gene and Efficacy of an ALK Kinase Inhibitor in Lung Cancer. *Clinical Cancer Research* 14, 4275–4283 (2008).
2. Choi, Y. L. et al. Identification of Novel Isoforms of the EML4-ALK Transforming Gene in Non-Small Cell Lung Cancer. *Cancer Research* 68, 4971–4976 (2008).
3. Gainor, J. F. et al. Molecular Mechanisms of Resistance to First- and Second-Generation ALK Inhibitors in ALK-Rearranged Lung Cancer. *Cancer Discovery* 1–48 (2016). doi:10.1158/2159-8290.CD-16-0596

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