# Ba/F3 EML4-ALK Cell Line

Cat. No: KC-0101 Version 17081902



## Ⅰ. Cell Line Information

| | |
|---|---|
| Catalog number | KC-0101 |
| Cell line name: | Ba/F3 EML4-ALK 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 |
| 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

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

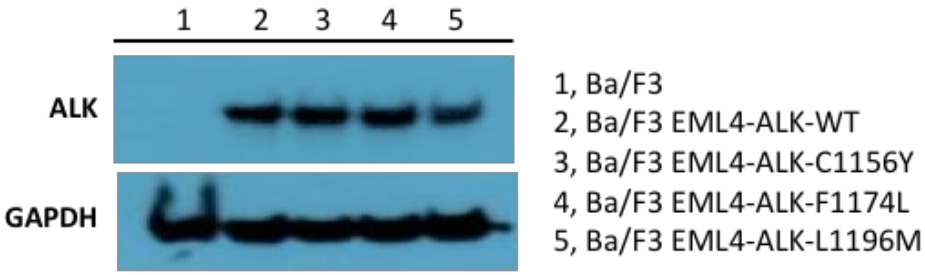
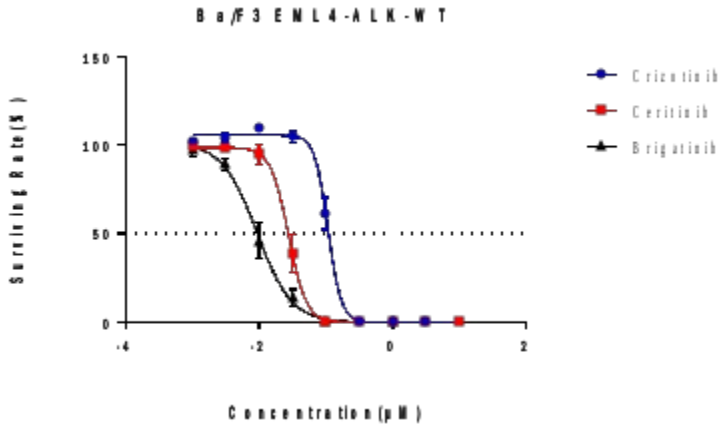



Figure: Characterization of ALK and its mutants overexpressing in Ba/F3 stable clones using Western Blot.

## 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 | 110.47 |
| Ceritinib | 27.43 |
| Brigatinib | 9.48 |

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 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 x$10^6$ 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. 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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