# Ba/F3 KIF5B-RET-V804M Cell Line

Cat. No: KC-1171 Version 19042301



## I. Cell Line Information

| | |
|---|---|
| **Catalog number** | KC-1171 |
| **Cell line name:** | Ba/F3 KIF5B-RET-V804M Cell Line |
| **Gene ID/Accession #:** | NA |
| **Host cell line** | Mouse Ba/F3 cell line |
| **Cell type:** | Pro-B cells |
| **Description:** | Stable Ba/F3 clone expressing exogenous KIF5B-RET fusion protein bearing V804M mutation in RET 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% 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 °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:** | BSL1 |
| **Storage:** | Liquid nitrogen immediately upon receiving |

## II. Background

RET, abbreviated for "rearranged during transfection" is a receptor tyrosine kinase for membranes of the gial cell line-derived neurotropic neurotrophic factor (GDNF) family of extracellular signaling molecules. Overactivation of RET have associated with several cancers. The identification of RET as a driver gene has led to the development of anticancer therapeutics agents.

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 KIF5B-RET-V804M cell line was generated using retrovirus vector expressing human KIF5B-RET-V804M sequence.

## IV. Characterization using Western Blot

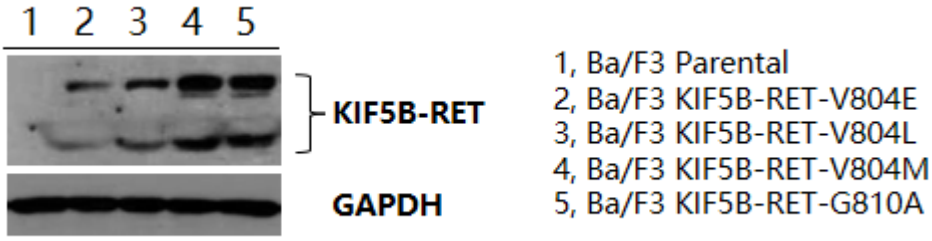
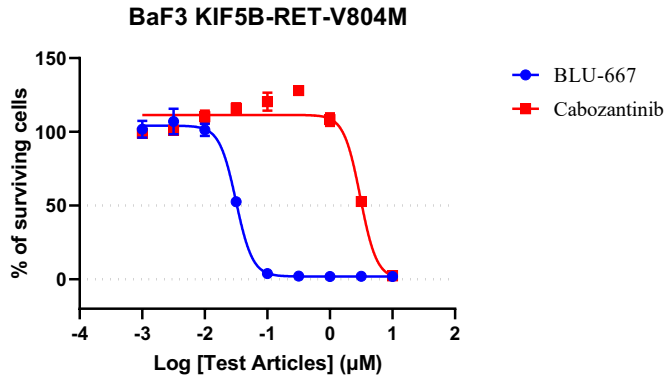



Figure: Characterization of KIF5B-RET and its mutants overexpressing in Ba/F3 stable clones.

## V. Application

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

**Example: kinase inhibitors screening**




**IC50 of RET inhibitor**

| Cell Line | IC50 ( μ M) |
|---|---|
| BLU-667 | 0.033 |
| Cabozantinib | 3.261 |

1. Harvest and seed the Ba/F3 cells expressing KIF5B-RET 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°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% $CO_2$ 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°C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

## VIII. References

1. Kawamoto Y, Takeda K, Okuno Y, et al. (2004). "Identification of RET autophosphorylation sites by mass spectrometry". J. Biol. Chem. 279 (14): 14213–24.
2. Rudin, Charles M, Alexander Drilon, and J T Poirier. 2014. “RET Mutations in Neuroendocrine Tumors: Including Small-Cell Lung Cancer.” Journal of Thoracic Oncology 9 (9). Elsevier: 1240–42.
3. Kohno, Takashi, Koji Tsuta, Katsuya Tsuchihara, Takashi Nakaoku, Kiyotaka Yoh, and Koichi Goto. 2013. “RET Fusion Gene: Translation to Personalized Lung Cancer Therapy.” Cancer Science 104 (11): 1396–1400.

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