

Ba/F3 KIF5B-RET-V804E Cell Line

Cat. No: KC-1169

Version 19042301

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I. Cell Line Information

Catalog number	KC-1169
Cell line name:	Ba/F3 KIF5B-RET-V804E 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 V804E 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% 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:	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-V804E cell line was generated using retrovirus vector expressing human KIF5B-RET-V804E sequence.

IV. Characterization using Western Blot

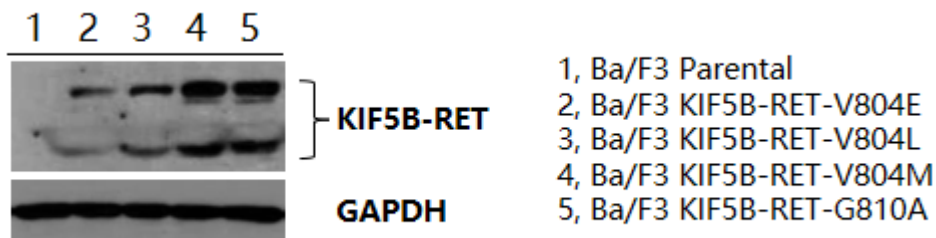
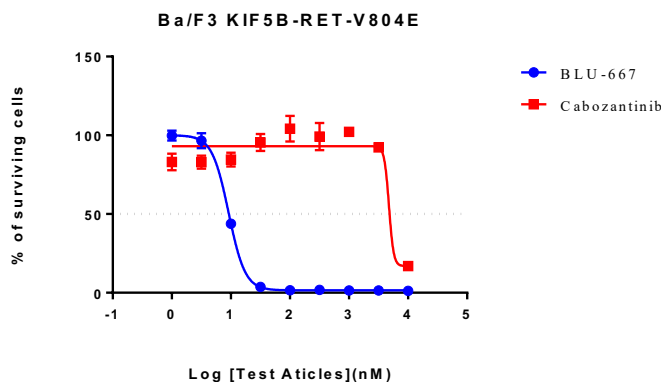


Figure: Characterization of KIF5B-RET and its mutants overexpressing in Ba/F3 stable clones.

V. Application

- Cell-based kinase inhibition screen
- Cell viability assay
- In vivo efficacy study

Example: kinase inhibitors screening



IC50 of RET inhibitor

Cell Line	IC50 (μM)
BLU-667	0.009
Cabozantinib	4.873

- Harvest and seed the Ba/F3 cells expressing KIF5B-RET mutant in 96-well plate (3000 cells/90ul medium).
- Next day, add 10ul 10X serially diluted compound solution each well and incubate the plates for another 72 hours.
- Add 100ul Cell Titer-Glo each well, mixed and readout using Envision.
- 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. 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.

Kyinno Co., Ltd.

Yizhuang Biomedical Park, No. 88, Beijing, China

Tel: +86-10-58222702

E-mail: bd@kyinno.com

Web: www.kyinno.com

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