

# Product Sheet

## H\_CCDC6-RET(V804M) BaF3 Cell Line

Catalog number: GM-C31367

Version 3.3.1.250117

CCDC6-RET kinase protein is a fusion protein composed of Coiled-Coil Domain-Containing Protein 6 (CCDC6) and Rearranged during Transfection (RET) kinase. This protein is mutated in certain tumor cells, leading to its activation and promoting tumor growth and metastasis. CCDC6-RET kinase protein is commonly found in tumors such as lung cancer and thyroid cancer, making it a potential therapeutic target. Research indicates that inhibiting the activity of CCDC6-RET kinase can effectively suppress tumor growth and spread, providing new directions for cancer treatment. There are various tyrosine kinase inhibitors (TKIs) targeting the CCDC6-RET fusion gene, including Cabozantinib, Selpercatinib, and Pralsetinib.

BA/F3 cells are a precursor B cell line that is dependent on interleukin-3 (IL-3), and certain protein kinases can substitute for IL-3 to support the growth of BA/F3 cells. This allows for the use of inhibitors to antagonize this effect, making it possible to study kinase inhibitors.

H\_CCDC6-RET(V804M) BaF3 Cell Line is a clonal stable BaF3 cell line constructed using non-viral transfection, constitutive expression of the CCDC6-RET gene. Can be used for the development and validation of small molecule drugs targeting RET.

### Specifications

<b>Quantity</b>	5E6 Cells per vial, 1 mL
<b>Product Format</b>	1 vial of frozen cells
<b>Shipping</b>	Shipped on dry ice
<b>Storage Conditions</b>	Liquid nitrogen immediately upon receipt

<b>Recovery Medium</b>	RPMI 1640+10% FBS+1% P.S
<b>Growth medium</b>	RPMI 1640+10% FBS+1% P.S+0.25 µg/mL Puromycin
<b>Note</b>	None
<b>Freezing Medium</b>	90% FBS+10% DMSO
<b>Growth properties</b>	Suspension
<b>Growth Conditions</b>	37°C, 5% CO <sub>2</sub>

**Mycoplasma Testing** The cell line has been screened to confirm the absence of Mycoplasma species.

**Safety considerations**      Biosafety Level 2

**Note**      It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

## Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Puromycin	Genomeditech/ <a href="#">GM-040401</a>
Pralsetinib (BLU-667)	Selleck/S8716
Cabozantinib (BMS-907351)	Selleck/S1119
Selpercatinib (LOXO-292)	Selleck/S8781
GMTiter™ Luminescent Cell Viability Assay	Genomeditech/ <a href="#">GM-040504</a>

## Figures

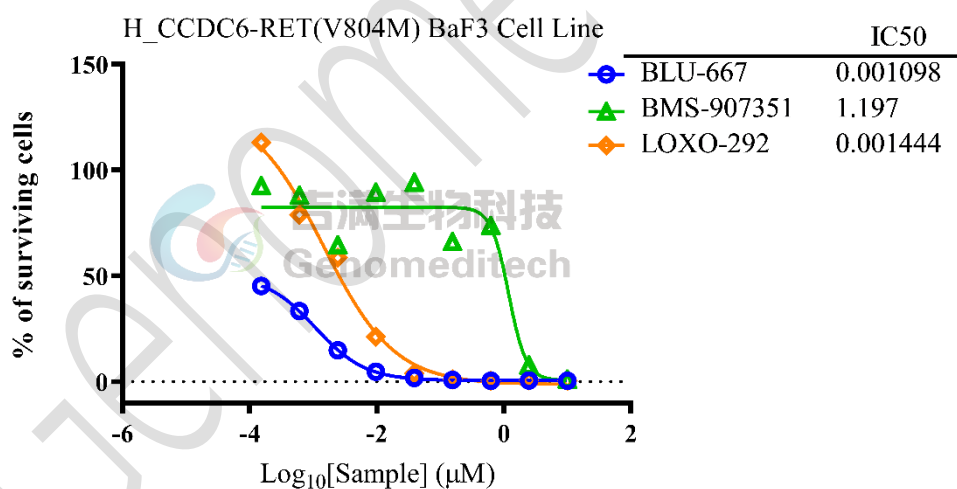


Figure 1 | Cell proliferation assay. The H\_CCDC6-RET(V804M) BaF3 Cell Line (Cat. GM-C31367) at a concentration of 1E4 cells/well (96-well format) was treated with serial dilutions of Pralsetinib (BLU-667) (Selleck/S8716), Cabozantinib (BMS-907351) (Selleck/S1119), Selpercatinib (LOXO-292) (Selleck/S8781) in assay buffer (RPMI 1640 + 10% FBS + 1% P.S) for 72 hours. He firefly luciferase activity was measured the GMTiter™ Luminescent Cell Viability Assay (Cat. [GM-040504](#)).



Figure 2 | The CCDC6-RET mutation analysis by Sanger sequencing.

## Cell Recovery

Recovery Medium: RPMI 1640+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at  $-70^{\circ}\text{C}$ . Storage at  $-70^{\circ}\text{C}$  will result in loss of viability.

- Thaw the vial by gentle agitation in a  $37^{\circ}\text{C}$  water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
- Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
- Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium. And spin at approximately  $176 \times g$  for 5 minutes. Discard supernatant.
- Resuspend cell pellet with the recommended complete medium. And dispense the suspension into 1-2 T-25 culture flasks.
- Incubate the culture at  $37^{\circ}\text{C}$  in a suitable incubator. A 5%  $\text{CO}_2$  in air atmosphere is recommended if using the medium described on this product sheet.

## Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- Centrifuge at  $176 \times g$  for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to  $5 \times 10^6$  cells/mL.
- Aliquot 1 mL into each vial.
- Place the vials in a controlled-rate freezing container and store at  $-80^{\circ}\text{C}$  for at least 1 day, then transfer to liquid nitrogen as soon as possible.

## Cell passage

Growth medium: RPMI 1640+10% FBS+1% P.S+0.25  $\mu\text{g}/\text{mL}$  Puromycin

Approximately 48-72 hours after the initial thawing, the cells can be passaged for the first time. After this initial passage, the culture medium can be adjusted to growth medium supplemented with antibiotics. If cells are not passaged within 48 hours, it is recommended to add some fresh recovery medium and place the flask horizontally.

- When the cell density reaches 1 - 1.2E6 cells/mL, subculture the cells. Do not allow the cell density to exceed 1.4E6 cells/mL.
- It is recommended to use T-25 flasks for subculturing.
- These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.
- During passaging, you can directly add fresh growth medium to the culture flask, gently pipette to resuspend the cells, and then transfer the cell suspension to a new T-25 flask for continued culture.

**Subcultivation Ratio: Maintain cultures at a cell concentraion between 3E5 and 1E6 viable cells/mL.**

**Medium Renewal: Every 2 to 3 days**

## Notes

- These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.
- During the first passage, pay attention to the nutrient supply; if not subculturing, make sure to add fresh recovery medium every other day as needed.

## Related Products

RET BAF3	
<a href="#">H_CCDC6-RET BaF3 Cell Line</a>	<a href="#">H_CCDC6-RET(V804L) BaF3 Cell Line</a>
<a href="#">H_KIF5B-RET BaF3 Cell Line</a>	<a href="#">H_KIF5B-RET(G810A) BaF3 Cell Line</a>
<a href="#">H_KIF5B-RET(G810S) BaF3 Cell Line</a>	<a href="#">H_KIF5B-RET(L) BaF3 Cell Line</a>
<a href="#">H_KIF5B-RET(L730I) BaF3 Cell Line</a>	<a href="#">H_KIF5B-RET(M918T) BaF3 Cell Line</a>
<a href="#">H_KIF5B-RET(S819A) BaF3 Cell Line</a>	<a href="#">H_KIF5B-RET(S904F) BaF3 Cell Line</a>
<a href="#">H_KIF5B-RET(T729-L730insL) BaF3 Cell Line</a>	<a href="#">H_KIF5B-RET(V804E) BaF3 Cell Line</a>
<a href="#">H_KIF5B-RET(V804L) BaF3 Cell Line</a>	<a href="#">H_KIF5B-RET(V804M) BaF3 Cell Line</a>
<a href="#">H_KIF5B-RET(V804M-G810S) BaF3 Cell Line</a>	<a href="#">H_KIF5B-RET(Y806N) BaF3 Cell Line</a>

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