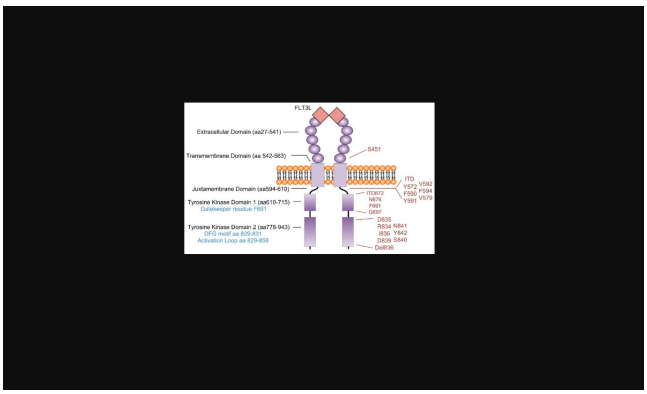

COBIOER BIOSCIENCES FLT3-ITD D835H-BaF3 Free Full-Text Targeting Oncogenic Signaling in Mutant Instructions

Contents

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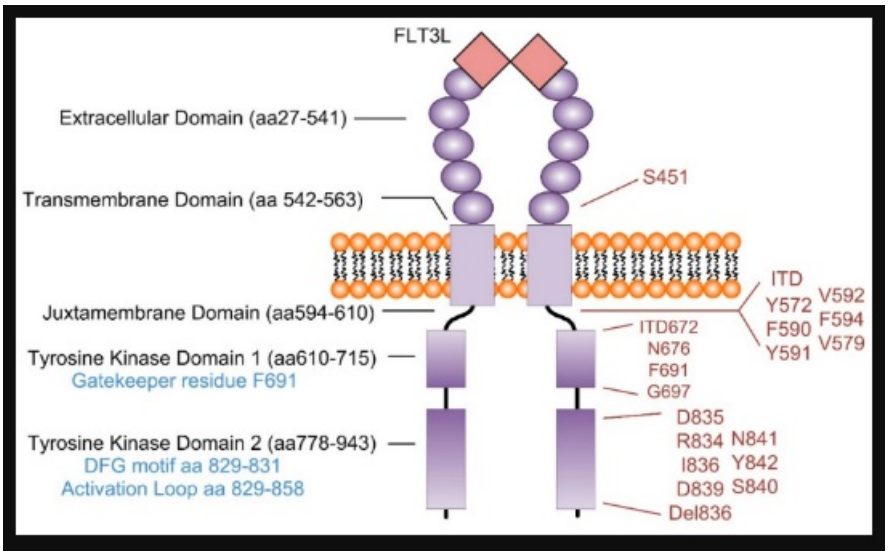

Product Information

The FLT3-ITD [D835H]/BaF3 CBP73239 is a stable cell line that expresses the FLT3 receptor-type tyrosine-protein kinase. The FLT3 protein activates Akt, Ras, and Erk pathways to regulate differentiation, proliferation, and survival of hematopoietic progenitor cells. The product is intended for anti-proliferation assays on hematologic tumors.

- Cell Line Name: FLT3-ITD [D835H]/BaF3 Host CellBa/F3
- Stability: 16 passages (in-house test, that not means the cell line will be instable beyond the passages we tested.)
- Freeze Medium: 90% FBS+10% DMSO
- Complete Culture Medium: RPMI-1640+10%FBS
- Mycoplasma Status: Negative

Product Usage Instructions

Materials to Be Supplied by the User

- Compounds to be tested
- RPMI-1640 (Gibco #C11875500CP)
- FBS (Gibco #10099-141C)

Protocol for the Cell Viability Assay

1. Culture the FLT3-ITD [D835H]/BaF3 CBP73239 cell line in the complete culture medium in a T75 flask at 37°C with 5% CO2.
2. When the cell density reaches between 3*105 and 2*106 viable cells/mL, subculture the cells by either adding fresh medium or replacing the medium.
3. For the anti-proliferation assay, plate 5,000 cells/well in a 96-well plate in 100 µL of RPMI-1640 medium containing 10% FBS and different concentrations of the test compounds.
4. Incubate the plate at 37°C with 5% CO2 for 72 hours.
5. Add 10 µL of Cell Counting Kit-8 (CCK-8) reagent to each well and incubate at 37°C with 5% CO2 for 4 hours.
6. Measure the absorbance at 450 nm using a microplate reader.

References

- **PMID:** 29316714
- **PMID:** 28538663
- **PMID:** 19467916
- **PMID:** 3018138

Introduction

- **Cell Line Name:** FLT3-ITD [D835H]/BaF3
- **Host Cell** Ba/F3
- **Stability:** 16 passages (in-house test, that not means the cell line will beinstable beyond the passages we tested.)
- **Freeze Medium:** 90% FBS+10% DMSO
- **Complete Culture Medium:** RPMI-1640+10%FBS
- **Mycoplasma Status:** Negative

Background

FLT3, receptor-type tyrosine-protein kinase FLT3, activates Akt, Ras, and Erk pathways to regulate differentiation, proliferation, and survival of hematopoietic progenitor cells (PMID: 29316714, PMID: 28538663). Activating mutations of FLT3 are common in hematologic tumors (PMID: 19467916) and the internal tandem duplication (ITD) mutation is commonly observed in acute myeloid leukemia (PMID: 30181385).

Representative Data

Anti-proliferation assay

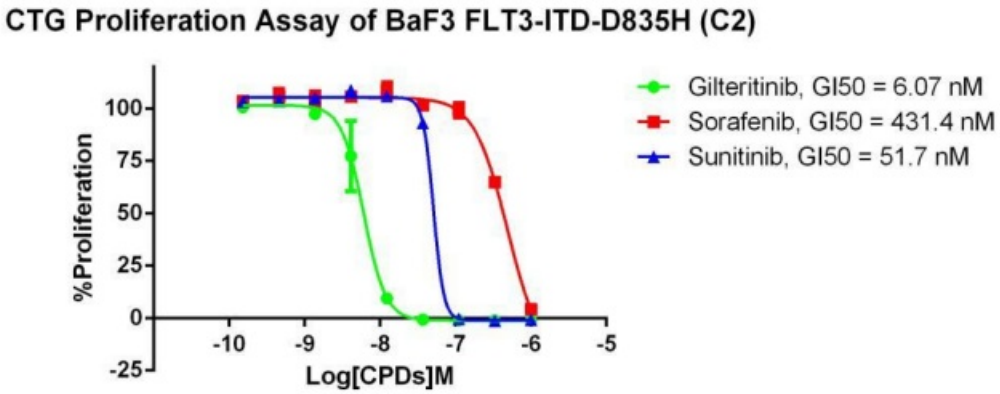

Figure 1. Anti-proliferation assay of three reference compounds on the FLT3-ITD [D835H]/BaF3 Stable Cell Line.

Handling Procedure for Flask Cultures

The flask was seeded with cells grown and completely filled with medium at Cobioer.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase contrast optics), carefully check for any evidence of microbial contamination.
2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 1000 rpm for 5 minutes.Remove shipping medium and save for reuse. Resuspend the cell pellet in 10ml of this medium.
3. From this cell suspension remove a sample for a cell count and viability.Adjust the cell density of the suspension to 3*10 5viable cells/ml in the shipping medium.
4. Incubate the culture, horizontally at 37°C in a 5%CO2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

Subculturing Procedure

- Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 3*10 5 viable cells/mL. Do not allow the cell density to exceed 2*10 6 cells/mL.
- **Interval:** Maintain cultures at a cell concentration between 3* 10 5 and2*10 6 viable cells/mL.
- **Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density)

Cryopreservation Procedure

1. Transfer cell suspension(4-8*10 6 cells) to a centrifuge tube and spinat approximately 1000rpm for 5 minutes.
2. Discard supernatant and resuspend cells in cryopreservation medium(90% FBS+10%DMSO).
3. Transfer the cells into Freezing Tube, 1ml/vial.
4. Place Freezing Tube in a Frosty container that is kept at room temperature and has sufficient is opropanol.
5. Place the Frosty container into the -80°C freezer overnight.
6. On next day, transfer the vials into liquid nitrogen.

Performing the Anti-proliferation Assay

1. Materials to Be Supplied by the User
    - Compounds to be tested
    - RPMI-1640 (Gibco #C11875500CP)
    - FBS (Gibco #10099-141C)
    - DMSO
    - CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Cat.No.: G7573)
    - 96 Well Assay Plate (White Plate, Clear Bottom with Lid Tissue Culture Treated Polystyrene 1/Pack, Corning #3610)
    - T25 Flask
    - 96 Well Storage Microplate(Corning#3357)
    - Synergy H1 Hybrid Multi-Mode Reader(Biotek)
2. Protocol for the Anti-proliferation Assay
    1. Take logarithmic growth cells, centrifuge and discard the culture supernatant, resuspend the centrifuged cells in fresh RPMI-1640+10%FBS medium.

2. Inoculate the resuspended cells into a 96-well cell culture plate with a white wall and transparent bottom, 100ul/well of cell suspension, inoculate two culture plates, and place in a 37°C cell incubator for overnight culture.
3. The next day, take one of the 96-well plates inoculated with cells, add100ul/well cell titer glo detection reagent and leave it for 60 minutes, read the value, and define it as G0 data.
4. Take another parallel plate, and take 11.1 ul/well of compound from the previously diluted 10*compound concentration dilution plate to this plate , And set up another DMSO control well, continue to incubate ina37°C cell incubator for 72 hours.
5. Take out the 96-well plate that has been treated with the compound for 72 hours from the incubator, add 100ul/well cell titer glo detection reagent and leave it for 60 minutes, read the value, and define it as G3data.
6. Calculate the cell proliferation rate corresponding to each well according to the following formula %Proliferation = (Test compoundwell G3-G0 average value) / (DMSO control well G3 average value-G0average value)*100.
7. According to the corresponding proliferation rate and concentration of each gradient concentration hole, use Prism Graphpad5.0 software tofit the gradient curve of cell proliferation, and calculate the GI50 of the compound (GI50 is defined as the corresponding compound concentration when the cell proliferation rate is 50%, The fitting formula in the software is as follows: Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)).

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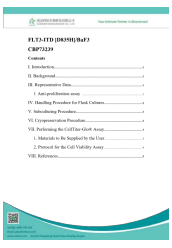
Documents / Resources

	COBIOER BIOSCIENCES FLT3-ITD D835H-BaF3 Free Full-Text Targeting Oncogenic Signaling in Mutant [pdf] Instructions FLT3-ITD D835H-BaF3 Free Full-Text Targeting Oncogenic Signaling in Mutant, FLT3-ITD D835H-BaF3, Free Full-Text Targeting Oncogenic Signaling in Mutant, Full-Text Targeting Oncogenic Signaling in Mutant, Text Targeting Oncogenic Signaling in Mutant, Targeting Oncogenic Signaling in Mutant, Oncogenic Signaling in Mutant, Signaling in Mutant, Mutant

References

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