Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_KRAS(G12L) BaF3 Cell Line

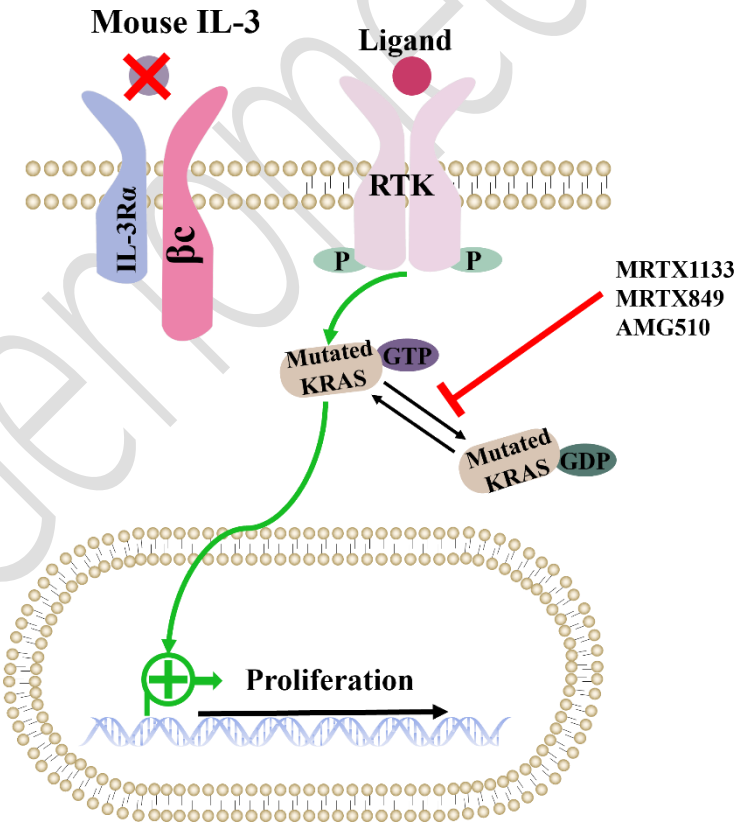
Catalog number: GM-C29490

Version 3.3.1.241220

KRAS is the most frequently mutated oncogene in human cancer, belonging to the RAS family. RAS is a GTPase protein that associates with the plasma membrane (PM) and acts as a switch between an active state bound to guanosine triphosphate (GTP) and an inactive state bound to guanosine diphosphate (GDP). Mutated KRAS can impair the GTPase activity of RAS proteins, locking them in their active state, which leads to the aberrant activation of several signaling pathways, ultimately resulting in uncontrolled cell growth and proliferation, invasiveness, angiogenesis, and metastasis.

BA/F3 cells are interleukin-3 (IL-3)-dependent precursor B cells, and certain protein kinases can substitute for IL-3 to promote the growth of BA/F3 cells. By using inhibitors to antagonize this effect, this system can be utilized for studies of kinase inhibitors.

H_KRAS(G12L) BaF3 Cell Line is a clonal stable BaF3 cell line constructed using lentiviral technology, constitutive expression of the KRAS gene. Can be used for the development and validation of small molecule drugs targeting KRAS.



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Specifications

Quantity	5E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Recovery Medium	RPMI 1640+10% FBS+1% P.S
Growth medium	RPMI 1640+10% FBS+1% P.S+0.25 μg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Suspension
Growth Conditions	37°C, 5% CO_2
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/GM-040401
96 Well Clear V-Bottom Tissue Culture	Corning/3894
96 well round well culture plate	NEST/701001
96 well White Flat Bottom Polystyrene Not Treated Microplate	Corning/3912
MRTX1133	BioChemPartner/BCP43012
MRTX849	BioChemPartner/BCP31538
AMG510	BioChemPartner/BCP33368
GMTiter™ Luminescent Cell Viability Assay	Genomeditech/GM-040504

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Figures

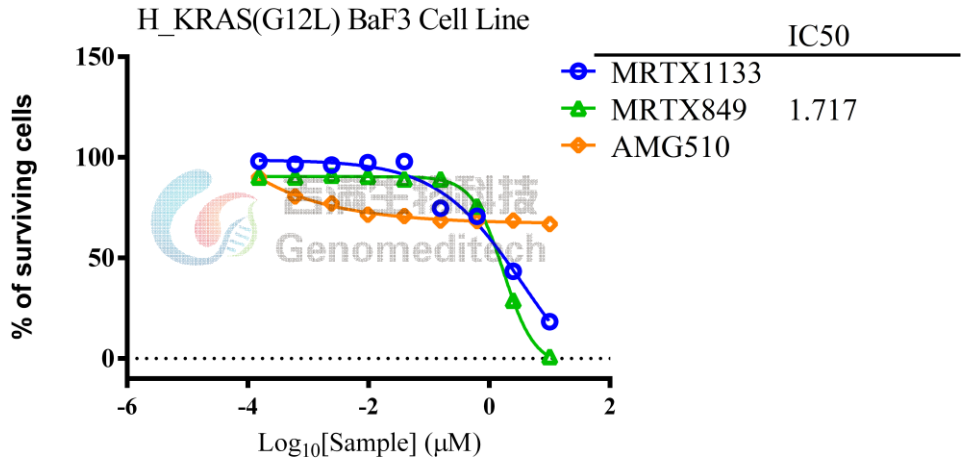

Figure 1 | Cell proliferation assay. The H_KRAS(G12L) BaF3 Cell Line (Cat. GM-C29490) at a concentration of 1E4 cells/well (96-well format) was treated with serial dilutions of MRTX1133 (BioChemPartner/BCP43012), MRTX849 (BioChemPartner/BCP31538), AMG510 (BioChemPartner/BCP33368) in assay buffer (RPMI 1640+10% FBS+1% P.S) for 72 hours. The firefly luciferase activity was measured using the GMTiter™ Luminescent Cell Viability Assay (Cat. GM-040504).

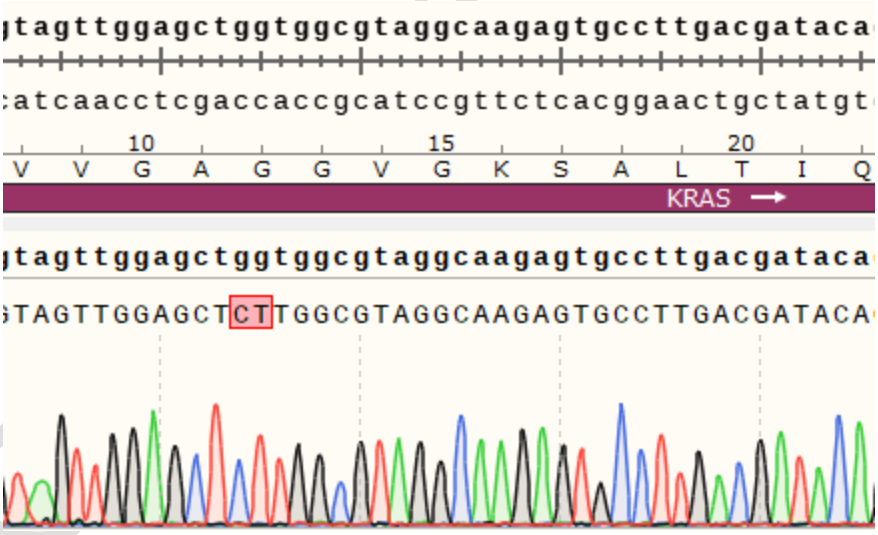

Figure 2 | The KRAS mutation analysis by Sanger sequencing.

Cell Recovery

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

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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°C. Storage at -70°C will result in loss of viability.

a) Thaw the vial by gentle agitation in a 37°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).
b) 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.
c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium. And spin at approximately 176 x g for 5 minutes.Discard supernatant.
d) Resuspend cell pellet with the recommended complete medium. And dispense the suspension into 1-2 T-25 culture flasks.
e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

a) Centrifuge at 176 x g for 3 minutes to collect cells.
b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
c) Aliquot 1 mL into each vial.
d) Place the vials in a controlled-rate freezing container and store at -80°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 μg/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.

a) 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.
b) It is recommended to use T-25 flasks for subculturing.
c) These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.
d) 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

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Notes

a) These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.

b) 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

KRAS BAF3	
H_KRAS(G12C) BaF3 Cell Line	H_KRAS(G12C-A59S) BaF3 Cell Line
H_KRAS(G12C-A59T) BaF3 Cell Line	H_KRAS(G12C-G12F) BaF3 Cell Line
H_KRAS(G12C-H95D) BaF3 Cell Line	H_KRAS(G12C-H95Q) BaF3 Cell Line
H_KRAS(G12C-H95R) BaF3 Cell Line	H_KRAS(G12C-Q99L) BaF3 Cell Line
H_KRAS(G12C-R68M) BaF3 Cell Line	H_KRAS(G12C-R68S) BaF3 Cell Line
H_KRAS(G12C-Y96C) BaF3 Cell Line	H_KRAS(G12C-Y96D) BaF3 Cell Line
H_KRAS(G12D) BaF3 Cell Line	H_KRAS(G12V) BAF3 Cell Line
H_KRAS(G13D) BaF3 Cell Line	H_KRAS(G13E) BaF3 Cell Line

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