Ba/F3 KRAS-G12D Cell Line

Cat. No: KC-1259 Version 19091701

Ⅰ. Cell Line Information

Catalog number	KC-1259
Cell line name:	Ba/F3 KRAS-G12D Cell Line
Gene ID/Accession #:	NM_033360.3
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous KRAS bearing G12D amino acid mutation
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:	1
Storage:	Liquid nitrogen immediately upon receiving

Ⅱ. Background

K-ras was the first identified oncogene in cellular genome, KRAS protein can bind GDP/GTP and possess intrinsic GTPase activity, plays an important role in the regulation of cell proliferation, the overactivation due to mutation can lead to continuously cell proliferation, and eventually develop into cancer.

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 KRAS-G12D cell Line was generated using retrovirus vector expressing human KRAS-G12D sequence.

IV. Characterization

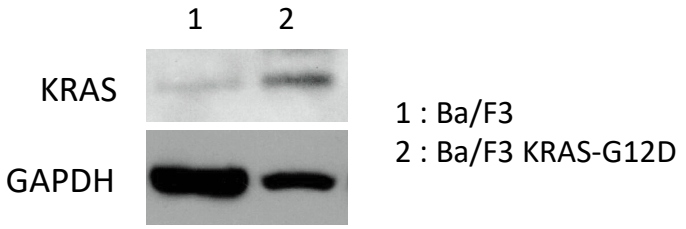


Figure: Characterization of KRAS and its mutants overexpressing in Ba/F3 stable clone

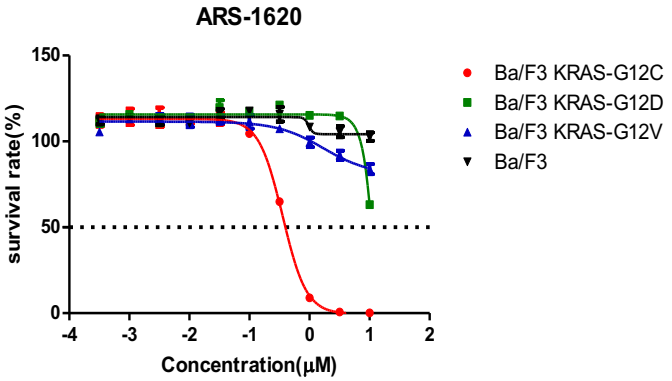
V. Application

a. Cell-based kinase inhibition screen

b. Cell viability assay

c. In vivo efficacy study

Example: kinase inhibitors screening



IC50 of KRAS inhibitor (ARS-1620)

Cell Name	IC50 (nM)
Ba/F3 KRAS-G12D	396.54
Ba/F3 KRAS-G12D	>10000
Ba/F3 KRAS-G12V	>10000
Ba/F3 Parent Cell	NA

1. Harvest and seed the Ba/F3 cells expressing KRAS 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 x10^6 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. Suchida N, Ryder T, Ohtsubo E (1982). "Nucleotide sequence of the oncogene encoding p21 transforming protein of Kirsten murine sarcoma virus". Science. 217 (4563): 937–939.
2. Ostrem, Jonathan M, Ulf Peters, Martin L Sos, James A Wells, and Kevan M Shokat. 2013. “K-Ras(G12D) Inhibitors Allosterically Control GTP Affinity and Effector Interactions.” Nature 503 (7477). Nature Publishing Group: 548–51. doi:10.1038/nature12796.
3. Janes, Matthew R, Jingchuan Zhang, Lian-Sheng Li, Rasmus Hansen, Ulf Peters, Xin Guo, Yuching Chen, et al. 2018. “Targeting KRAS Mutant Cancers with a Covalent G12D-Specific Inhibitor.” Cell 172 (3). Elsevier Inc.: 578–581.e17.

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