

Ba/F3 HER2 Cell Line

Cat. No: KC-0134

Version 17033103

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I. Cell Line Information

Catalog number	KC-0134
Cell line name:	Ba/F3 HER2 Cell Line
Gene ID/Accession #:	NM_004448.3
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous HER2 gene.
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:	1
Storage:	Liquid nitrogen immediately upon receiving

II. Background

HER2, also named ERBB2, is a cell-surface receptor tyrosine kinase, ERBB2 overexpression or overactivity have associated with a number of cancers, especially the breast cancer. The identification of ERBB2 as a driver gene has led to the development of anticancer therapeutics agents, including lapatinib, Neratinib and Herceptin.

Compound	IC50 (uM)
Lapatinib	0.039
Neratinib	0.004

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 HER2 cell Line was generated using Retroviral vector expressing human HER2 sequence.

IV. Characterization using Western Blot

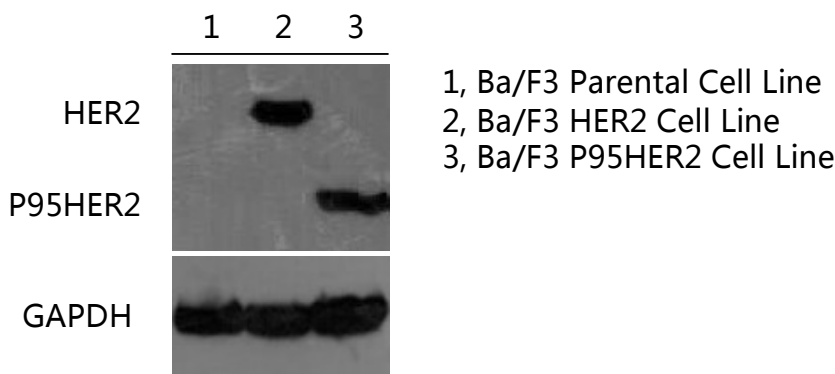
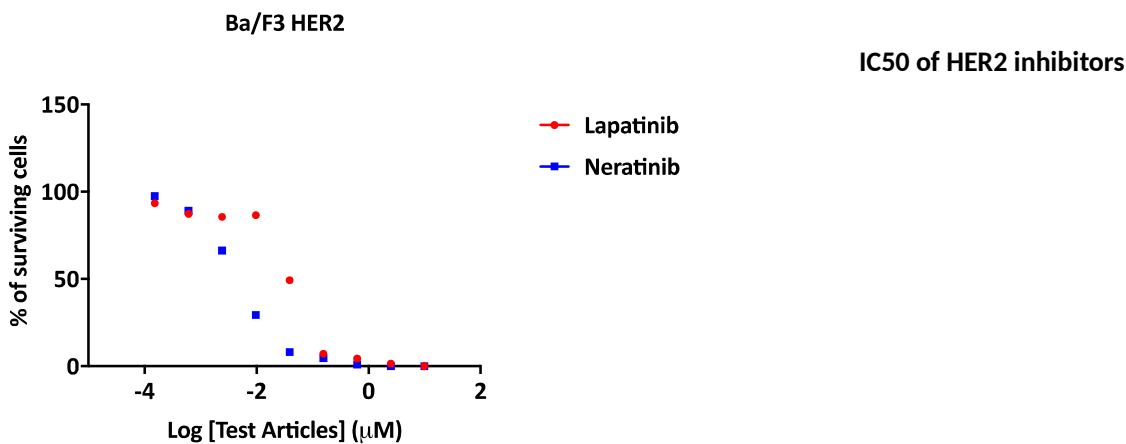


Figure: Characterization of HER2 and its mutants overexpressing in Ba/F3 stable clones using Western Blot.

V. Application

- a. Cell-based kinase inhibition screen
- b. Cell viability assay
- c. In vivo efficacy study

Example: kinase inhibitors screening



1. Harvest and seed the Ba/F3 cells expressing HER2 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 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. Wang, Shizhen Emily, Archana Narasanna, Marianela Perez-Torres, Bin Xiang, Frederick Y Wu, Seungchan Yang, Graham Carpenter, Adi F Gazdar, Senthil K Muthuswamy, and Carlos L Arteaga. 2006. "HER2 Kinase Domain Mutation Results in Constitutive Phosphorylation and Activation of HER2 and EGFR and Resistance to EGFR Tyrosine Kinase Inhibitors.." *Cancer Cell* 10 (1). Elsevier: 25-38.
2. Robichaux, Jacquelyne P, Yasir Y Elamin, Zhi Tan, Brett W Carter, Shuxing Zhang, Shengwu Liu, Shuai Li, et al. 2018. "Mechanisms and Clinical Activity of an EGFR and HER2 Exon 20-Selective Kinase Inhibitor in Non-Small Cell Lung Cancer." *Nature Medicine*, April. Springer US, 1-15.

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