Description

EGFR Ba/F3 Cell Line is a mouse pro-B cell line (Ba/F3) in which human EGFR (epidermal growth factor receptor) is expressed under the control of an EF1a promoter. This cell line was generated by transduction of Ba/F3 cells with EGFR Lentivirus (BPS Bioscience #82459).

This cell line has been validated by flow cytometry, as well as viability assays demonstrating its sensitivity to EGFR inhibitors.

Background

EGFR (epidermal growth factor receptor), also known as ERBB-1 and HER1, is the cell-surface tyrosine kinase receptor for members of the epidermal growth factor family. Its ligands include EGF, TGFα (transforming growth factor alpha), HB-EGF (heparin-binding EGF), betacellulin, amphiregulin, epiregulin and epigen. EGFR exists as an inactive monomer until it gets activated. Upon ligand binding it forms a homo- or heterodimer, for instance with HER2 (human epidermal growth factor receptor 2), which induces autophosphorylation, creating binding sites for adaptor proteins such as GRB2 (growth factor receptor-bound protein 2) and/or CBL (Casitas B-lineage lymphoma). EGFR can bind to several adaptor proteins simultaneously and thus activate multiple positive and negative signaling pathways. Overexpression and/or hyperactivation of EGFR kinase is associated with several human cancers such as lung, glioblastoma (GBM), and epithelial tumors of the neck and head, being the most common mutation in GBM and breast cancer. Mutations in EGFR can result in constantly activated EGFR, allowing tumor cell proliferation and development of resistance to drugs. Its role in cancer has led to the development of anticancer therapeutics targeting EGFR. There are several clinically approved inhibitors, such as Erlotinib and Gefitinib, for the treatment of NSCLC (non-small cell lung cancer) and pancreatic cancer. In addition, several monoclonal antibodies have also been approved, namely Cetuximab. Patients that respond to treatment to anti-EGFR therapy tend to develop resistance later on, highlighting the need for further detailed studies into the role of this protein and new therapeutic avenues.

Ba/F3 cells are murine pro-B cells, and their proliferation is dependent on interleukin-3 (IL-3) signaling. However, expression of some constitutively active tyrosine kinases (e.g., BCR-ABL and some EGFR mutants) allows Ba/F3 cells to grow independently of IL-3. Because Ba/F3 cells expressing these kinases depend on kinase signaling for growth in the absence of IL-3, these cells are a useful model system for studying kinase oncogenes and screening tyrosine kinase inhibitors.

Application

- Useful for testing EGFR inhibitors.
- Use as a wildtype control when testing mutant-selective EGFR inhibitors.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

Ba/F3, mouse IL-3-dependent pro-B cell line, suspension.

Mycoplasma Testing

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

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
EGF Recombinant	BPS Bioscience #90201

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
EGF Recombinant	BPS Bioscience #90201
LIVE-Step™ Cell Assay System	BPS Bioscience #82648
White, clear-bottom 96-well tissue culture plate	Corning #3610
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note:
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Complete Thaw Media

Thaw Medium 2 and human epidermal growth factor (EGF):

RPMI 1640 (ATCC modification) medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 80 ng/ml human EGF.

Media Required for Functional Cellular Assay

Complete Thaw Media

Thaw Medium 2 and human epidermal growth factor (EGF):

RPMI 1640 (ATCC modification) medium supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 80 ng/ml human EGF.

Note: Mouse IL-3 is essential for maintenance of parental Ba/F3 cells. In the absence of mouse IL-3, the EGFR Ba/F3 cell line is dependent on signaling through the EGFR pathway for cell survival. ***It is recommended to grow this cell***

line in Complete Thaw Medium (containing EGF). *In the absence of IL-3, EGFR expression becomes heterogeneous across the cell population, however these cells can be used to test EGFR inhibitors as their cell viability depends on EGFR activity in these conditions.*

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Complete Thaw Medium.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Complete Thaw Medium.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Complete Thaw Medium and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Complete Thaw Medium.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.1 x 10^6 cells/ml in Complete Thaw Medium. The sub-cultivation ratio should maintain the cells between 0.1 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

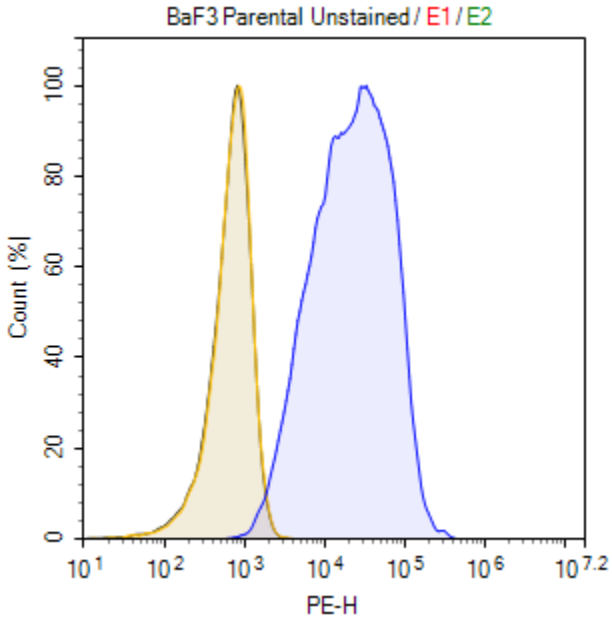

Figure 1: Expression of EGFR in EGFR Ba/F3 Cell Line (cultured with 80 ng/ml human EGF) by flow cytometry.
Cells were stained with PE-conjugated anti-human EGFR Antibody [clone AY13] and analyzed by flow cytometry. Parental Ba/F3 cells are shown in yellow, unstained parental Ba/F3 cells are shown in gray, and the EGFR Ba/F3 cells are shown in blue. The y axis shows the % of cells, while the x-axis represents the fluorophore intensity.

Functional Validation: EGFR Ba/F3 Cell Line viability assay

- This experiment measures the IC_{50} of EGFR inhibitors against wild-type EGFR.
- All samples and controls should be performed in triplicate.
- The assay should include "Treated", "No Treatment" and "Background Luminescence" conditions.
- Avoid plating cells at the outer edges of the plate.

1. From a cell culture growing in Complete Thaw Medium (**no mouse IL-3**), seed the cells at a density of 10,000 cells/well in 50 µl of Complete Thaw Medium into a white, clear-bottom 96-well cell culture plate. Keep a few wells without cells as "Background Luminescence" control.

   Note: Do not seed cells in the wells on the outer edges of the plate.

2. Prepare a 3-fold increment serial dilution of an EGFR inhibitor at concentrations 2-fold higher than the desired final concentrations (50 µl/well) in Complete Thaw Medium.

3. Add 50 µl of each dilution to the "Treated" wells.

4. Add 50 µl of Complete Thaw Medium to the "No Treatment" wells.

5. Add 100 µl of Complete Thaw Medium to "Background Luminescence" wells.

6. Add 250 µl of Complete Thaw Medium to the wells on the outer edges of the plate to minimize edge effects due to evaporation.

7. Incubate at 37°C with 5% CO_2 for 72 hours.

8. Add 100 µl/well of LIVE-Step™ reagent (#82648).

9. Incubate at room temperature for ~15 minutes.

10. Measure luminescence using a luminometer.

11. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The percentage of viable cells is the background-subtracted luminescence of treated cells divided by the background-subtracted luminescence of untreated control wells x 100.

$$\%\ viable\ cells = 100 \times \frac{(luminescence\ of\ treated\ cells - avg\ background)}{(avg\ luminescence\ of\ untreated\ cells - avg\ background)}$$

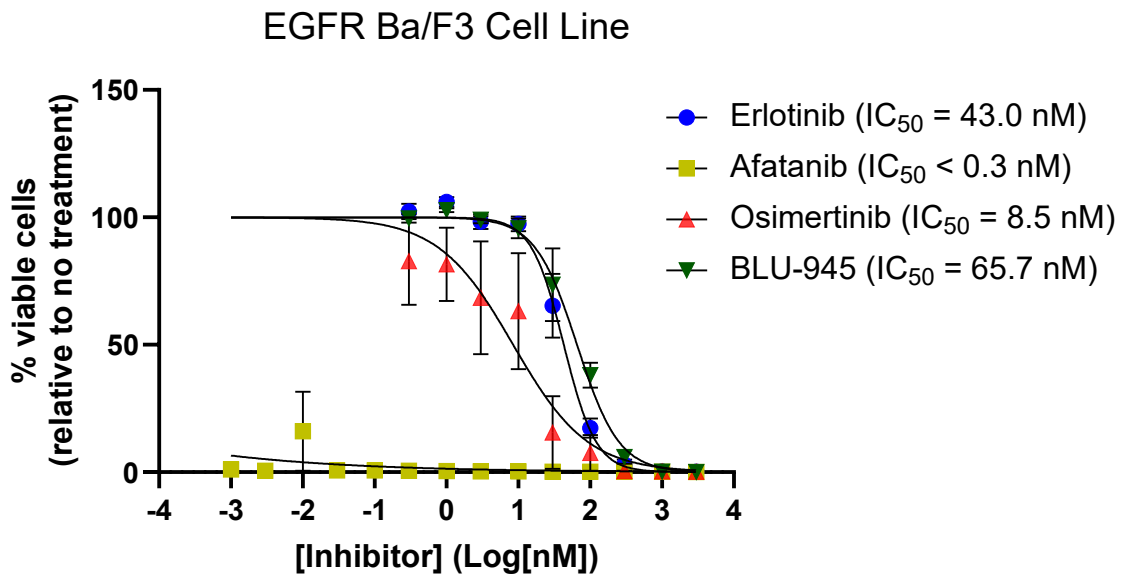

Figure 2: Dose-dependent response of EGFR Ba/F3 Cell Line to EGFR inhibitors (n = 3 independent experiments).

EGFR Ba/F3 cells were grown in the presence of hEGF, which activates EGFR, and incubated with increasing concentrations of the EGFR inhibitors Erlotinib (1st generation), Afatinib (2nd generation), Osimertinib (3rd generation) and BLU-945 (4th generation, GLPBIO #GC63910) for 72 hours. Cell viability was measured using the LIVE-STEP™ Cell Assay System (#82648). In this assay, luciferase activity is proportional to the number of metabolically active, viable cells, and is measured using a luminometer. The results are shown as percent cell viability relative to the no-treatment control. For each curve, each point represents the mean between independent experiments, and error bars represent the standard error. The nonlinear regression dose-response curve used to calculate the IC_{50} values is shown. This graph shows the mean of 3 independent experiments for each inhibitor, each testing 9 different inhibitor concentrations as well as a no treatment control. Each data point shown on the graph represents the mean and standard error for 1-3 measurements, depending on the range of concentrations tested in each experiment.

Results are representative.

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References

Jiang J., *et al.*, 2005 *Cancer Res* 65(19):8968-8974.
Nakamura J.L., 2007 *Expert Opin. Ther. Targets* 11(4):463-472.
Parada Y., *et al.*, 2001 *J. Biol. Chem.* 276(26):23572-23580.
Uribe M.L., *et al.*, 2021 *Cancers (Basel)* 13(11):2748.

Related Products

Products	*Catalog #*	*Size*
EGFR (L858R-T790M-C797S) Ba/F3 Cell Line	82464	2 vials
EGFR Lentivirus	82459	2 vials
EGFR A549 Cell Line	82477	2 vials

Version 030626