Description

EGFR A549 Cell line is a human lung cancer cell line (A549) in which the endogenous human epidermal growth factor receptor (EGFR) is not expressed due to CRISPR/Cas9 editing, and where wildtype EGFR is now expressed under the control of an EF1a promoter. This cell line was generated by transduction of EGFR Knockout A549 cells (BPS Bioscience #83541) with EGFR Lentivirus (BPS Bioscience #82459). It was designed as a an appropriate control for EGFR mutant cell lines (BPS Bioscience #82478 and #82480).

This cell line has been validated by flow cytometry.

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. 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 anti-EGFR therapy tend to develop resistance, highlighting the need for further studies and new therapeutic avenues.

Application

- Useful as a control when studying phenotypes related to EGFR mutations found in the EGFR (L858R-T790M-C797S) A549 Cell Line (BPS Bioscience #82478) or EGFR (Exon19del) A549 Cell Line (BPS Bioscience #82480).

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

A549 is a human lung alveolar vessel carcinoma cell line. Adherent epithelial cells.

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 6	BPS Bioscience #60183
Growth Medium 6C	BPS Bioscience #78077

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: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37℃ 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

Thaw Medium 6 (BPS Bioscience #60183):
DMEM supplemented with 10% FBS and 1% Penicillin/Streptomycin.

Growth Medium 6C (BPS Bioscience #78077):
DMEM supplemented with 10% FBS, 1% Penicillin/Streptomycin, and 0.25 µg/ml of Puromycin.

Cell Culture Protocol

Note: A549 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 Thaw Medium 6.

   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 Thaw Medium 6.

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 attachment and viability. Change medium to fresh Thaw Medium 6 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Replace media every 2-3 days until cells reach 90% confluency. At first passage and subsequent passages, use Growth Medium 6C.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Thaw Medium 6 and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 6C.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:2 to 1:10 once or twice per week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 6C and count the cells.

3. 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.

4. 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.

5. 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

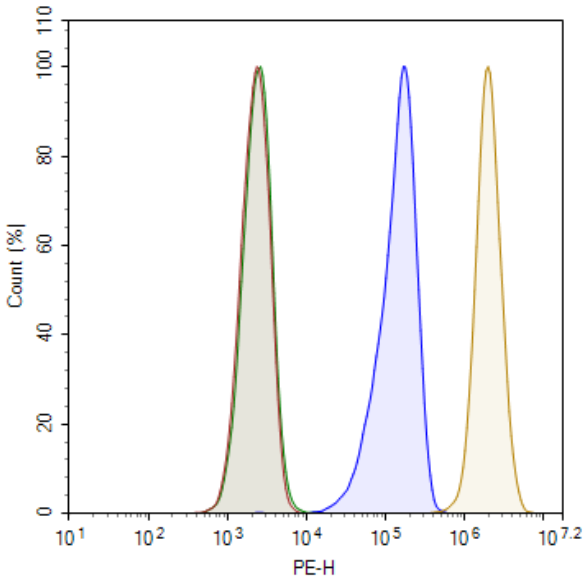

Figure 1: Expression of EGFR in EGFR A549 Cell Line by flow cytometry.
Cells were stained with PE-labeled anti-human EGFR Antibody [clone AY13] (BioLegend #352904) and analyzed by flow cytometry. A549 cells are shown in blue, unstained A549 cells are shown in green, EGFR Knockout A549 cells (#83541) are shown in red, and the EGFR A549 cells are shown in yellow. The y-axis shows the % of cells, while the x-axis represents the fluorophore intensity.

Results are representative.

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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.

Notes
The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

References
Liu J-Y., *et al.*, 2024 *Oncol. Lett.* 29(3):109.
Nakamura J.L., 2007 *Expert Opin. Ther. Targets* 11(4):463-472.
Uribe M.L., *et al.*, 2021 *Cancers (Basel)* 13(11):2748.

Related Products

Products	*Catalog #*	*Size*
EGFR (L858R-T790M-C797S) A549 Cell Line	82478	2 vials
EGFR (Exon19del) A549 Cell Line	82480	2 vials
EGFR Knockout A549 Cell Line	83541	2 vials
EGFR Lentivirus	82459	500 µl x 2

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