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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

EGFR Knockout A549 Cell line is an A549 lung cancer cell line in which human EGFR (epidermal growth factor receptor) is no longer expressed due to CRISPR/Cas9 genome editing. This cell line was generated by electroporation of A549 cells with ribonucleoprotein complexes of Cas9 and sgRNAs targeting human EGFR.

This cell line has been validated by genomic sequencing and 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 an asymmetric dimer, 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.

Application

- Use as a negative control when testing EGFR inhibitors or EGFR-directed biologics in A549 cells.
- Study phenotypes resulting from EGFR knockout.

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

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.
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 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

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 Thaw Medium 6.

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 Thaw Medium 6.

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 Thaw Medium 6 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

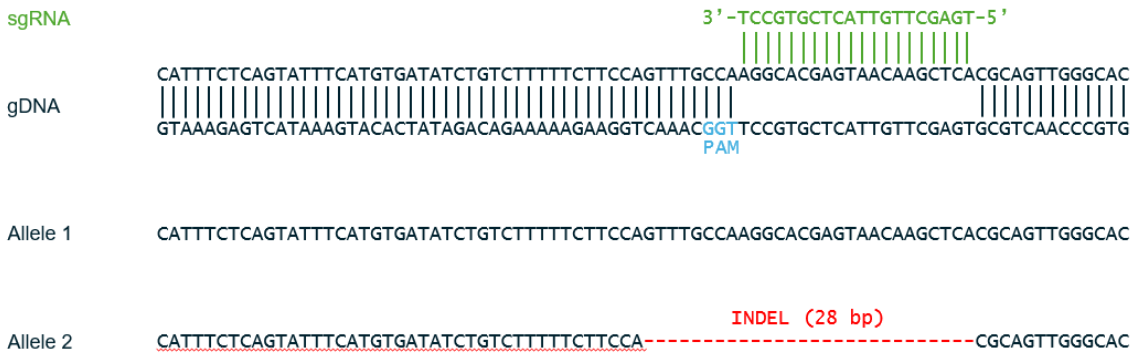

Figure 1: Genomic sequencing of EGFR Exon 2 in the EGFR Knockout A549 Cell Line.
Genomic DNA from the EGFR Knockout A549 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (single guide RNA) is shown in green, and the Indel (Insertion/Deletion) in one of the two EGFR alleles is highlighted in red. The EGFR genomic DNA is labeled as gDNA.

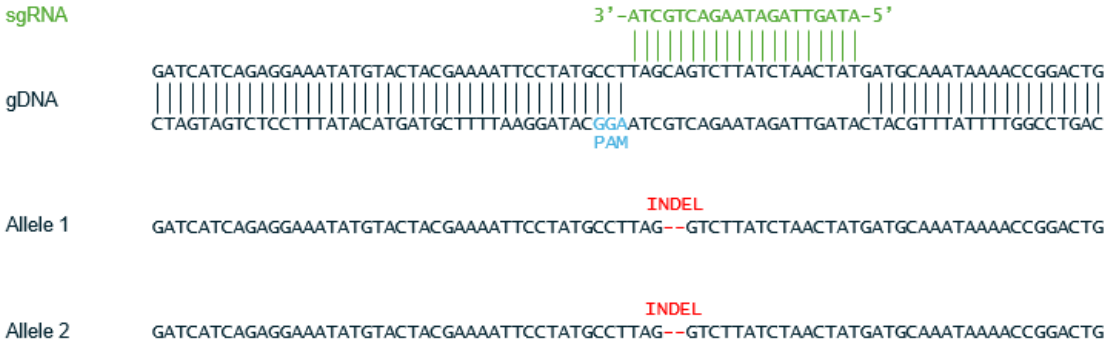

Figure 2: Genomic sequencing of EGFR Exon 3 in the EGFR Knockout A549 Cell Line.
Genomic DNA from the EGFR Knockout A549 cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (single guide RNA) is shown in green, and the Indels (Insertions/Deletions) in the two EGFR alleles are highlighted in red. The EGFR genomic DNA is labeled as gDNA.

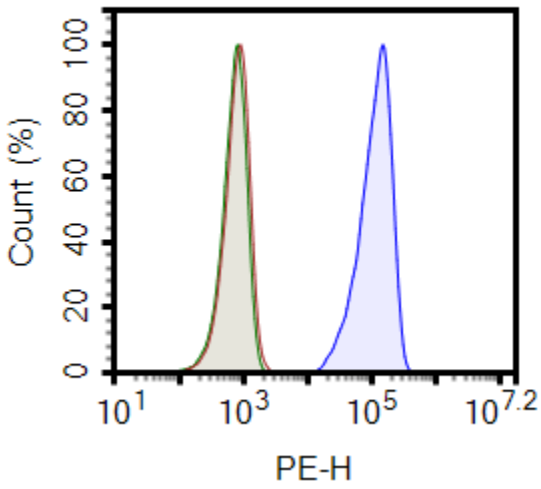

Figure 3: Expression of EGFR in EGFR Knockout A549 Cell Line by flow cytometry.
Cells were stained with PE anti-human EGFR Antibody (BioLegend #352904) and analyzed by flow cytometry. Parental A549 cells are shown in blue, unstained parental A549 cells are shown in green, and the EGFR Knockout A549 cells are shown in red. The y axis shows the % of cells, while the x-axis represents the fluorophore intensity.

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 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

Nakamura J.L., 2007 *Expert Opin. Ther. Targets* 11(4):463-472.
Uribe MK., *et al.*, 2021 *Cancers (Basel)* 13(11):2748.

Related Products

Products	*Catalog #*	*Size*
Human EGFRvIII CHO K1 Cell Line (High or Low expression)	78145	2 vials
Chemi-Verse™ EGFR Kinase Assay Kit	82504	96 reactions
EGFR(T790) Kinase Assay Kit	40323	96 reactions
EGFR(T790M, C797S) (del746-750) Kinase Assay Kit	78595	96 reactions
EGFR(L858R) Kinase Assay Kit	40324	96 reactions
Anti-FcGR3A-Anti-EGFR Bispecific Antibody	101535	50 µg

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