Technical Bulletin

A549 EGFR Antigen Panel Cell Lines

Catalog Number ATG002

Product Description

The A549 EGFR antigen panel is a series of three (3) genetically modified cell lines that targets the EFGR locus in A549 lung carcinoma cells.

The panel consists of the following cell lines (Figure 1):

1. ATG002A-1VL: A549 EGFR Knockout
2. ATG002B-1VL: A549 EGFR Low
3. ATG002C-1VL: A549 EGFR High

CompoZr® zinc finger nuclease (ZFN) technology was used to create a pan-allelic, targeted knockout (KO) of the EGFR gene in wild type A549 cells (Catalog Number 86012804-1VL). Generation of the A549 EGFR KO cell line was confirmed via next-generation sequencing (NGS) analysis as shown in Figure 2 and flow cytometry as shown in Figure 4.

Following single cell cloning and expansion of the A549 EGFR KO cell line, MISSION® lentiviral particles were used to randomly integrate and express exogenous, full-length EGFR cDNA at low or high levels relative to endogenous expression in the parental A549 cell line to generate the A549 EGFR Low (ATG002B-1VL) and A549 EGFR High (ATG002C-1VL) cell lines.

EGFR protein expression was measured in the antigen panel via fluorescent-activated cell sorting (FACS) as shown in Figure 4.

Figure 1
Generation of the Tumor-Associated Antigen Panel Cell Lines

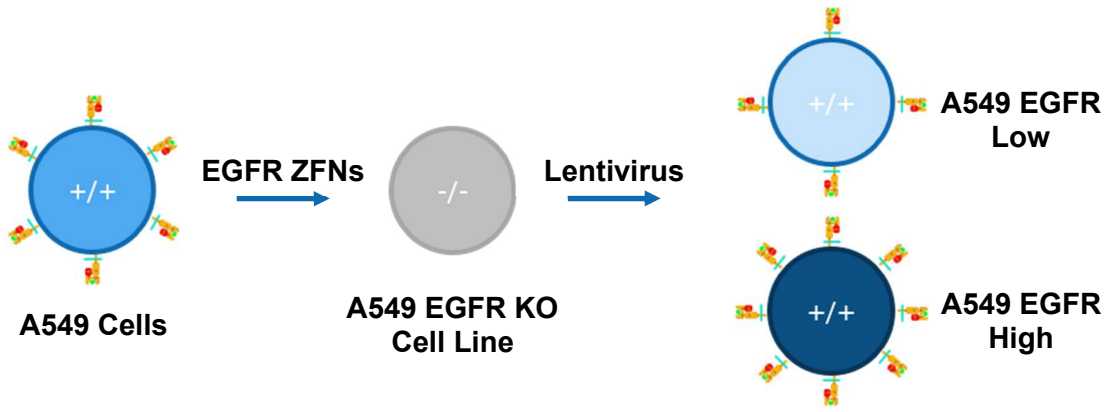


Figure 2
Genomic mutations in the EGFR locus.
Site-specific pan-allelic -8 deletion and +2/+4 bp insertions at the EGFR locus in A549 cells

Top panel: wild type A549 cells, ZFN target site is highlighted in yellow.
Bottom panel: A549 EGFR KO cells (and subsequent EGFR low/EGFR high cells), ZFN target site is highlighted in yellow.

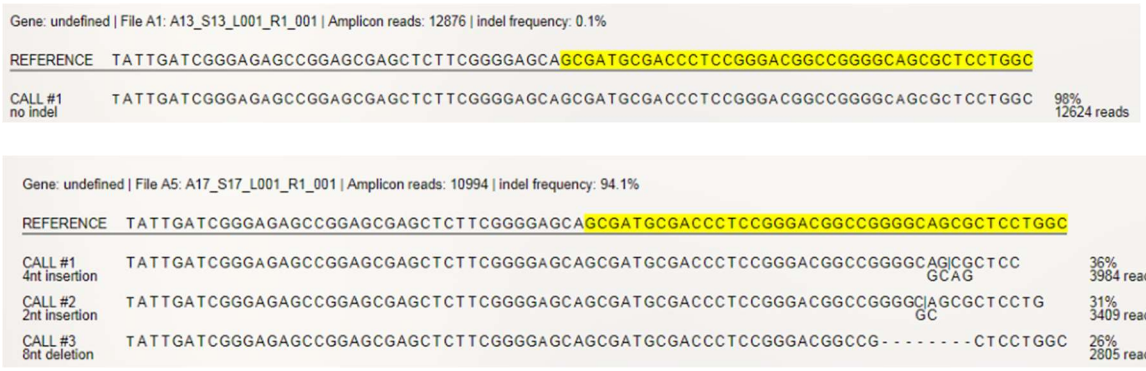
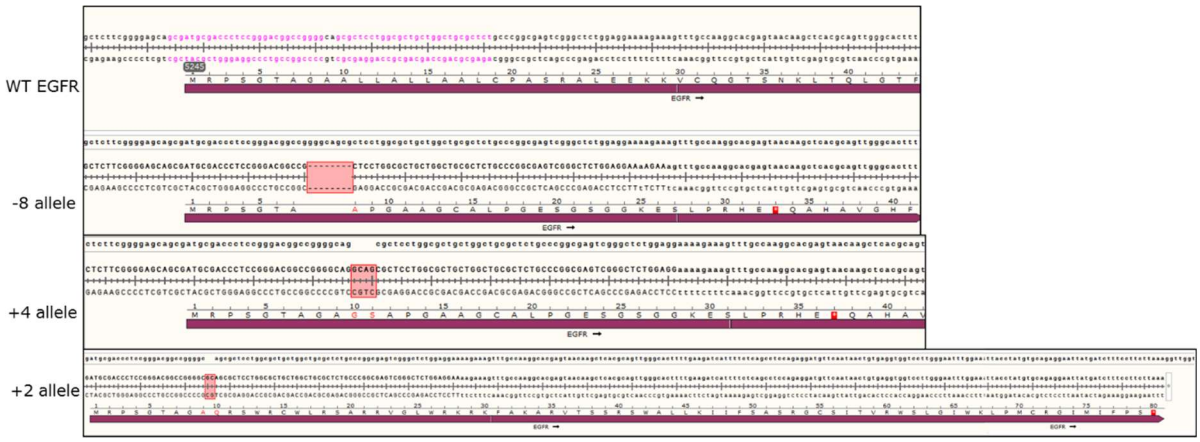


Figure 3. Exonic alignment of -8 deletion and +2/+4 insertions. Both the -8 deletion and +2/+4 insertions result in a predicted premature stop codon in exon 2 of human EGFR as indicated by a red asterisk.



Genomic sequence at the target region recognized by the ZFN pair.
GCGATGCGACCCTCCGGGACGGCCGGGGCAGCGCTCCTGGCGCTGCTGGCTGCGCTCT

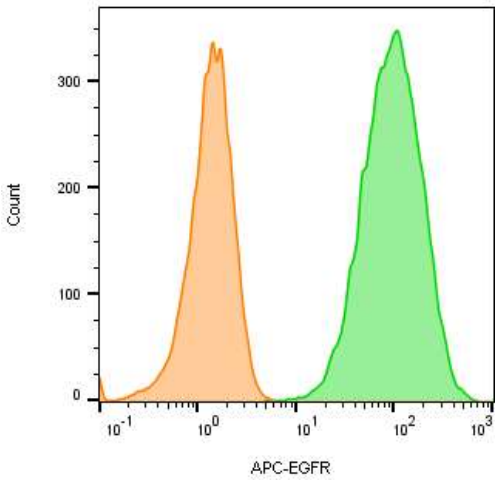
NGS PCR for knockout
Forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNcccgcacggtgtgagc
Reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNNNagacacgcccttacctttct

Wildtype amplicon sequence (314 bp)
CCCGCACGGTGTGAGCGCCCGACGCGGCCGAGGCGGCCGGAGTCCCGAGCTAGCCCCGGCGGCCGCCGC
CGCCCAGACCGGACGACAGGCCACCTCGTCGGCGTCCGCCCGAGTCCCCGCCTCGCCGCCAACGCCACAA
CCACCGCGCACGGCCCCCTGACTCCGTCCAGTATTGATCGGGAGAGCCGGAGCGAGCTCTTCGGGGAGCA
GCGATGCGACCCTCCGGGACGGCCGGGGCAGCGCTCCTGGCGCTGCTGGCTGCGCTCTGCCCGGCGAGT
CGGGCTCTGGAGGAAAAGAAAGGTAAGGGCGTGTCT

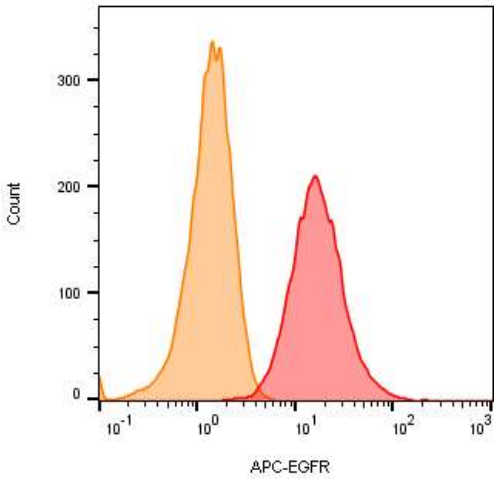
Figure 4
EGFR Expression in the A549 EGFR Antigen Panel via FACS Analysis

A549 cells were stained with an APC-conjugated EGFR antibody and analyzed by MacsQuant. Cells were gated by scatter, doublets were excluded, and only live cells were analyzed. Appropriate IgG controls were used and did not stain any of the cells (data not shown).

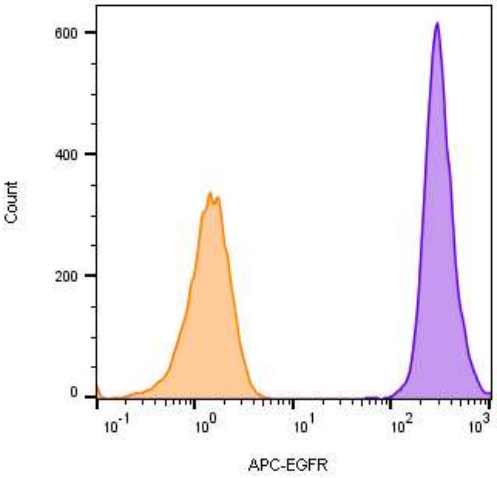
Orange: EGFR KO Cells
Red: EGFR Low Cells
Purple: EGFR High Cells
Green: Wild Type A549 Cells



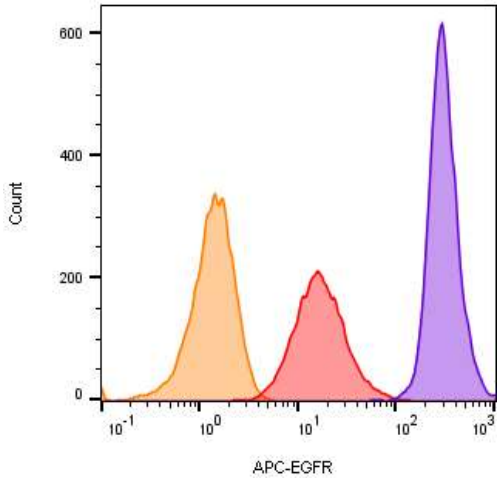
A549 EGFR KO cells (orange) compared to wild type A549 cells (green).



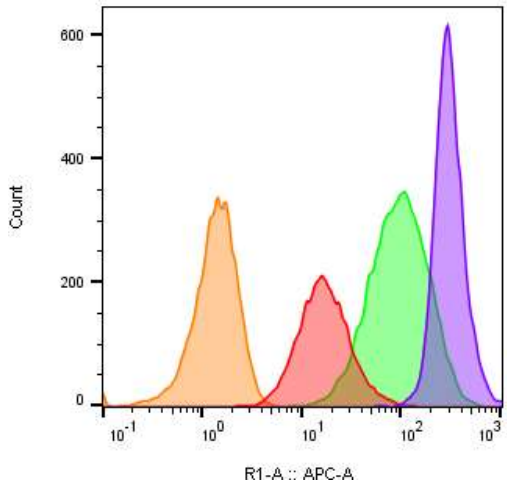
A549 EGFR Low cells (red) compared to A549 EGFR KO cells (orange).



A549 EGFR High cells (purple) compared to A549 EGFR KO cells (orange)



EGFR expression of the entire A549 EGFR antigen panel. KO (orange), low (red), high (blue).



EGFR expression in the A549 EGFR antigen panel cell lines compared to wild type A549 cells.

Components

This product is three (3) cryovials containing a minimum of 1 million A549 cells in each vial.

The cryoprotectant medium used is CryoStor® cell cryopreservation medium containing 10% DMSO (Catalog Number C2874).

Cell Line Description

Organism: Homo sapiens (human)
Tissue: Carcinoma; Lung
Gender: Male
Morphology: Epithelial
Growth Properties: Adherent

DNA Profile

STR-PCR Data:
Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 11
D16S539: 11,12
D18S51: 14,17
D21S11: 29
D3S1358: 16
D5S818: 11
D7S820: 8,11
D8S1179: 13, 14
FGA: 23
Penta_D: 9
Penta-E: 7,11
TH01: 8,9,3
TPOX: 8,11
vWA: 14

The STR profile of this cell line matches that of its parental cell line European Collection of Authenticated Cell Cultures (ECACC) Catalog Number 86012804. Please see the ECACC Catalog Number 86012804 datasheet for additional information about the origin of this cell line.

Reagents and Equipment Required but Not Provided

- Dulbecco's Modified Eagle's Medium, high glucose, Catalog Number D5796
- Fetal Bovine Serum, USA origin, sterile-filtered, Catalog Number F2442
- Trypsin-EDTA solution, 1×, Catalog Number T4049
- Hank's Buffered Saline Solution (HBSS), Catalog Number H6648
- Biological safety cabinet
- 70% ethanol (prepared from Ethanol, Catalog Number E7148)
- Bio-Pure™ alcohol wipes, Catalog Number Z688487
- 37 °C water bath (operating range 35-38 °C)
- Sterile 15 mL conical tubes
- Centrifuge
- Serological pipettor with 1, 2, 5, 10, and 25 mL sterile pipettes
- Vacuum aspiration system and sterile plastic or glass aspiration tips
- Sterile 25 cm^2 or 75 cm^2 culture flasks
- 37 °C, 5% CO_2 incubator

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Precaution: It is recommended that protective gloves and clothing always be used, and a full-face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to the gas phase may result in the rapid expansion of the vessel, potentially blowing off its cap with dangerous force creating flying debris.

Storage/Stability

Store cells at -196 °C (liquid nitrogen)

Upon receiving a shipment of frozen cells, it is important the end user gives the shipment attention without delay. To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and **not** at -70 °C. **Storage at -70 °C will result in loss of viability**.

At the time a cell line is ordered, end users should also consider the culture conditions for the new cell line and make sure the appropriate medium will be available when the cells arrive.

Procedure

Medium Preparation Instructions

The base medium for this cell line is DMEM High Glucose Medium (Catalog Number D5796)

Complete Medium: To make the complete growth medium, add Fetal Bovine Serum (Catalog Number F2442) to a final concentration of 10%.

Thawing of Frozen Cells

1. Thaw the vial by gentle agitation in a 37 °C water bath for ~1 minute. To reduce the possibility of contamination, keep the O-ring and cap out of the water.
2. Remove the vial from the water bath as soon as the contents are thawed and decontaminate by dipping in or spraying with 70% ethanol solution. All the operations from this point on should be carried out under aseptic conditions.
3. Transfer the cell suspension to a 15 ml conical tube containing 9 mL of warmed Complete Medium.
4. Centrifuge the cells at 125 × g for 5-7 minutes at room temperature.
5. Aspirate the media from the tube. Resuspend the cell pellet with 6 mL of warmed Complete Medium and plate into a 25 cm^2 or 75 cm^2 culture flask.
6. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested, prior to the addition of the vial contents, the culture vessel containing the Complete Medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6) and temperature (37 °C).
7. Incubate the culture at 37 °C in a 5% CO_2 in air atmosphere incubator.

Sub-culturing Procedure

Volumes used in the procedure are for a 75 cm^2 flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with HBSS (Catalog Number H6648)
3. Add 2.0-3.0 mL of Trypsin-EDTA solution to flask and incubate at 37 °C for 6 minutes to detach the cells.
4. Add 6.0-8.0 mL of Complete Medium and aspirate cells by gentle pipetting.
5. Add appropriate aliquots of the cell suspension into new culture vessels. Sub-cultivation ratio: 1:4 to 1:20.
6. Incubate cultures at 37 °C in an incubator containing an atmosphere of 5% CO_2 in air.

References

1. Giard, D.J., et al. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors., *J. Natl. Cancer Inst*., 51(5), 1417-23 (1973). PMID: 4357758

Additional product and technical information can be obtained by searching for the catalog number at sigmaaldrich.com.

These products are covered by the Purchase Agreement as described in Exhibit 1.

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Tumor-Associated Antigen Panel Cell Lines

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