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Product Information

A549 GFP-EGFR
Lung Carcinoma Cell Line with GFP-tagged EGFR

Catalog Number **CLL1141**
Storage Temperature –196 °C (liquid nitrogen)

TECHNICAL BULLETIN

Product Description
This product is an A549 cell line in which the genomic EGFR gene has been endogenously tagged with a Green Fluorescent Protein gene (GFP) using CompoZr® Zinc Finger Nuclease technology (ZFN). The cell line shows redistribution of EGFR from the cell membrane to the endosomes upon addition of EGF, making it useful for high content screening of compounds.

CompoZr technology is a fast and reliable way to manipulate the genome in a targeted fashion. ZFNs are synthetic proteins engineered to bind DNA at a sequence-specific location and create a double strand break (www.compozrzfn.com). The cell's natural machinery repairs the break in one of two ways: non-homologous end joining or homologous recombination. The homologous recombination pathway was utilized to insert a transgene into a desired target location – at the C-terminus of the EGFR locus. A donor construct containing a fluorescent reporter gene (GFP) flanked by sequences homologous to the cut site was nucleofected into the A549 cells along with ZFNs designed to cut near the genomic target site (Figures 1a and 1b). Integration resulted in endogenous expression of a fluorescent EGFR chimeric fusion protein. Knock-in cells were sorted into single cells by flow cytometry and expanded into clonal populations. Testing of these clones was used to select a single EGFR-GFP clone as a stable cell line (Figures 2a and 2b). Junction PCR showed that at least one allele is tagged with GFP (Figure 3a) and Southern analysis showed that there were no off-target insertions of the GFP (Figure 3b).

Receptor tyrosine kinases (RTKs), such as the epidermal growth factor receptor (EGFR), are cell-surface growth-factor receptors that dimerize and autophosphorylate upon ligand binding. They transduce signals across the cell membrane and thus regulate diverse cell functions.[1] Overexpression of EGFR has been found in various cancers. This has lead to the development of clinically approved therapeutics that target EGFR for colon, lung, breast, pancreatic and head and neck cancers.[2]

With ZFN-mediated gene tagging in this knock-in cell line, EGFR's native gene regulation is conserved resulting in normal levels of protein expression and preservation of protein function. In contrast to biochemical assays or immunostaining, using a tagged protein under endogenous regulation allows detection of RTK activation in live cell assays. In wild type cells, ligand-induced activation of EGFR leads to receptor internalization.[3] Thus, having a fluorescent tag on the EGFR protein allows visualization and tracking of this normal internalization process after the addition of EGF to the cells (Figure 4). These internalization kinetics can be quantified by image analysis software to count intracellular granules. Moreover, preservation of selectivity of this reporter response is demonstrated by data showing that the selective EGFR inhibitor, Tyrphostin AG 1478, abolishes the EGF-induced internalization of the receptor (Figure 4).

GFP and TagGFP2 are all synonymous for the fluorescent reporter gene in this document. The GFP used in this cell line originated from Evrogen, referred to as TagGFP2:
http://evrogen.com/products/TagFPs.shtml

For further information on our CompoZr modified cell lines go to the website:
www.wherebiobegins.com/biocells

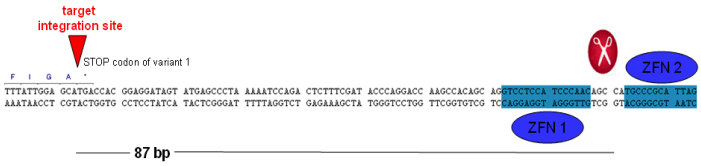
Component

A549 knock-in cell line with EGFR gene tagged on the C-terminus with GFP Catalog No. CLL1141	1 vial

1 vial contains ~2×10^6 cells in Cell Freezing Medium-DMSO 1×, Catalog No. C6164.

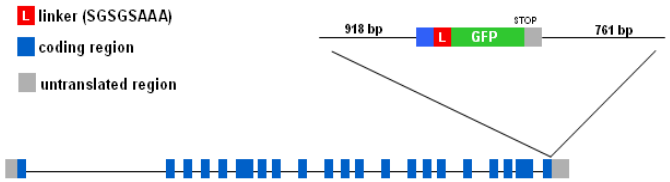
The design of tag sequence integration at the EGFR locus

Figure 1a.



Schematic of the genomic sequence at the target region for integration of the fluorescent GFP tag. DNA of EGFR, showing the start codon, CompoZr ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow).

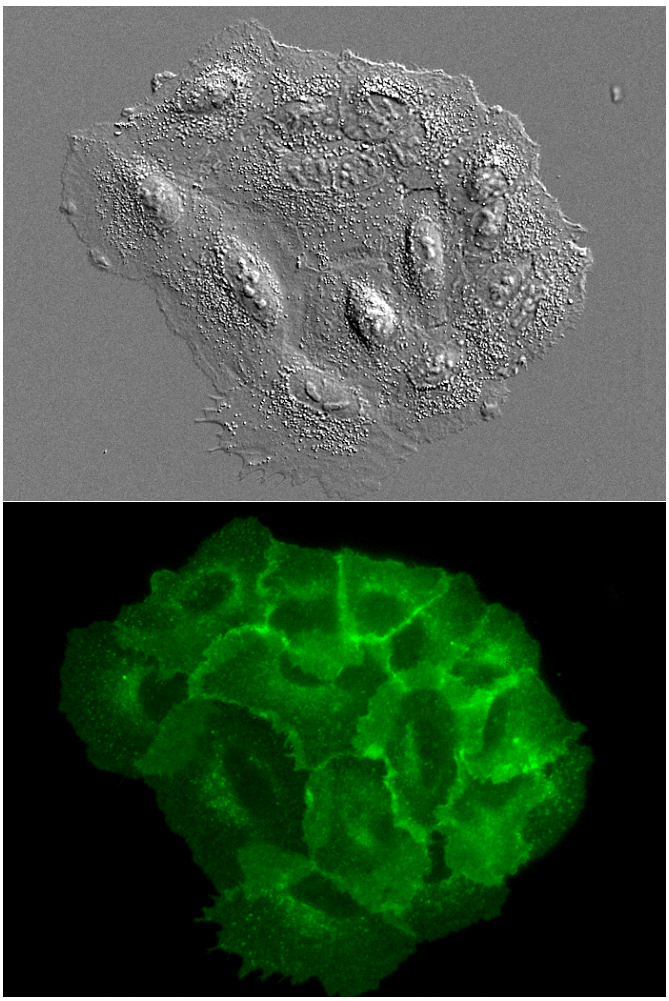
Figure 1b.



Schematic of the EGFR locus and the donor with the locus showing the coding regions (blue) and untranslated regions (gray). The donor (top) has the homology arms of indicated length and the GFP sequence (green) fused near the end of the EGFR coding sequence (a C-terminal fusion).

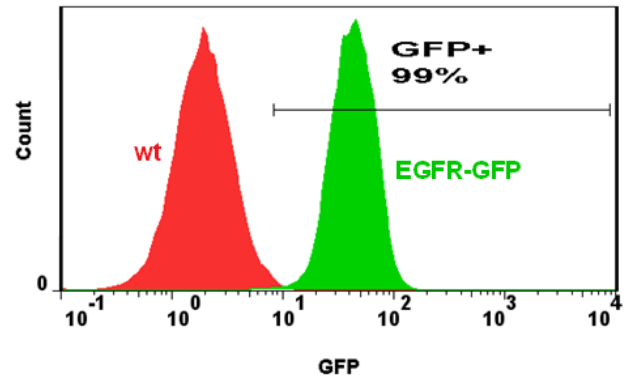
Localization & expression levels of endogenously tagged EGFR with GFP

Figure 2a.



Differential interference contrast (DIC) and fluorescence microscopy images of an isolated cell clone expressing endogenous EGFR protein tagged with GFP (ex 450–490/em 500–550, 40×/1.4 oil). The cells were imaged in Hanks balanced salt solution (Catalog No. H8264) supplemented with 2% fetal bovine serum (Catalog No. F2442). EGFR's endogenous expression levels allow for good fluorescence separation of the endogenously expressed EGFR-GFP fusion versus autofluorescence levels (see Figure 2b).

Figure 2b.

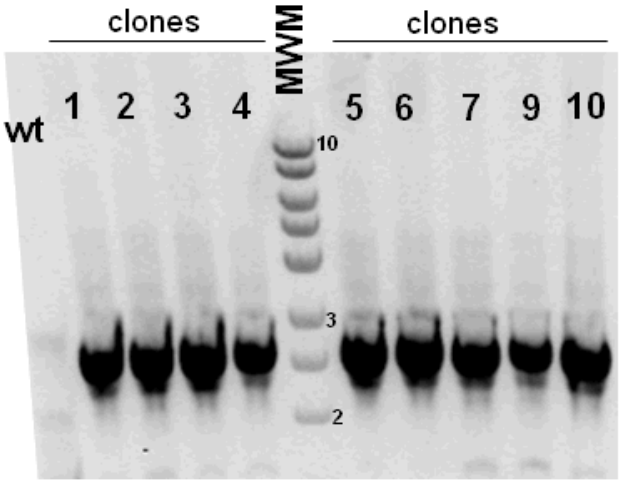


	Fluorescent Mean Intensity
A549 (wt)	1.9
EGFR-GFP in A549	42.0

Fluorescence analysis of the endogenous EGFR tagged with GFP clone compared to wild type A549 (autofluorescence) using MACS Quant from Miltenyi Biotec.

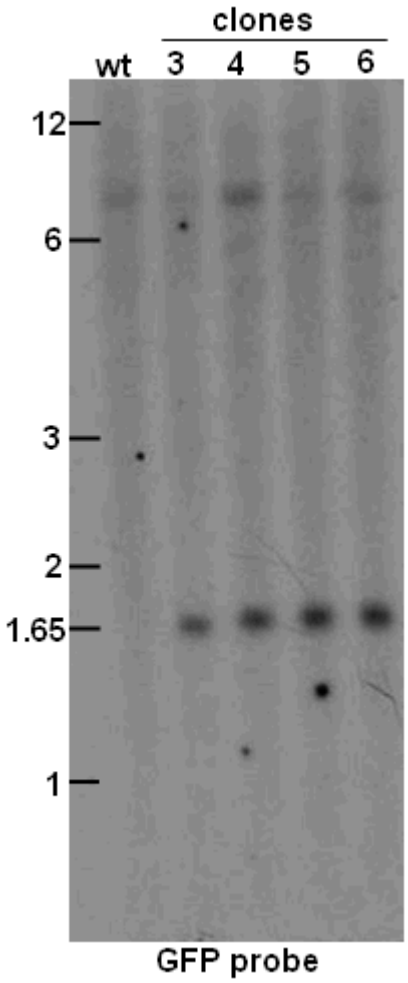
Molecular analysis to identify targeted integration in A549 endogenous EGFR tagged with GFP clones

Figure 3a.



Junction PCR using GFP forward and EGFR reverse primers produced a 2511bp characteristic fragment for targeted integration for all clones tested. No PCR product can be detected in the wild type control.

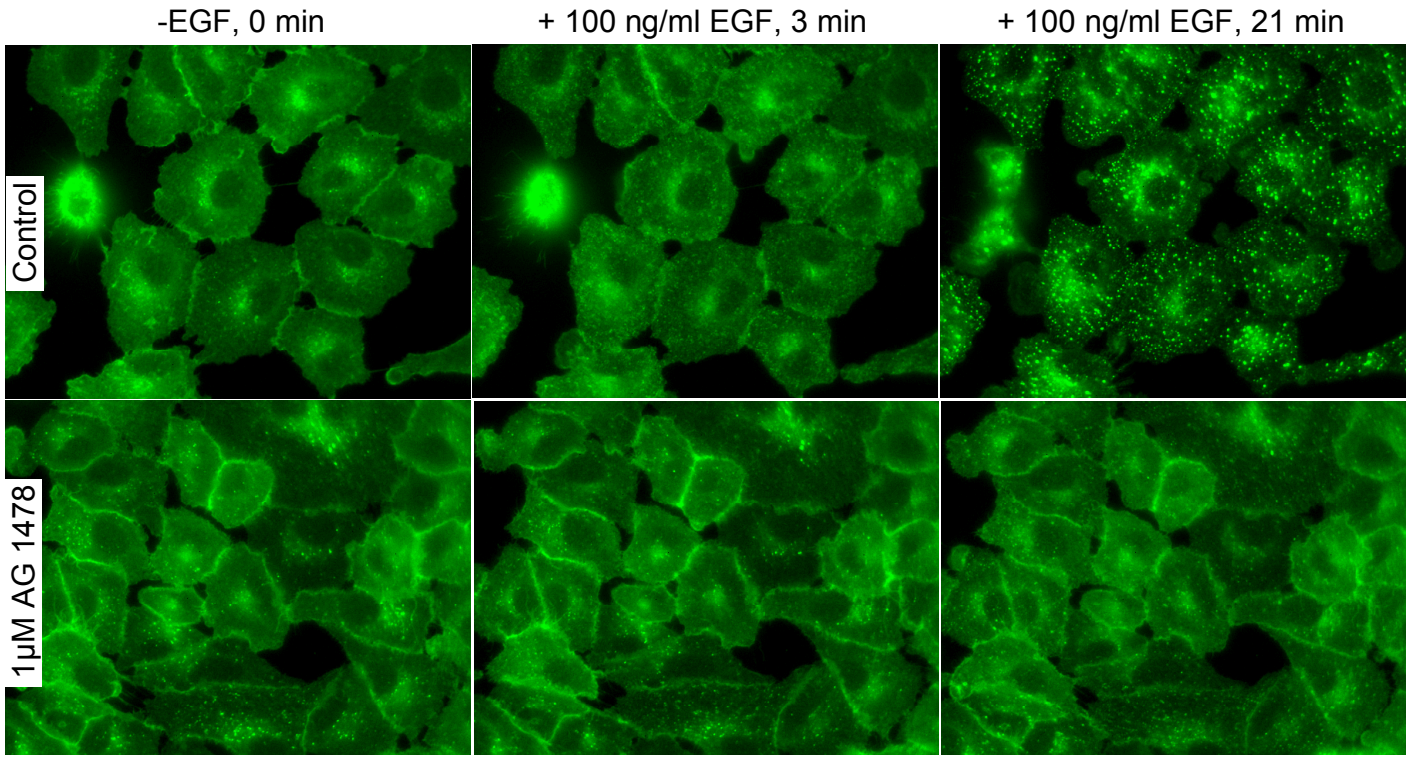
Figure 3b.



Southern blotting shows no random integration from the plasmid donor initially used to create the clones. Genomic DNA from single cell clones 3, 4, 5 and 6 were digested with BamHI and Hind III restriction endonucleases. Proper targeted insertion of the GFP into the EGFR locus should produce a hybridized band of approximately 1.5 Kb in size. Radioactive-labeled GFP probe was used. Clone 3 was chosen as the final product.

Translocation of endogenously expressed EGFR-GFP fusion protein from cell membrane to endosomes and inhibition of translocation by a selective EGFR inhibitor

Figure 4



Fluorescence microscopy analysis demonstrates that EGFR-GFP fusion protein is translocated from the plasma membrane to endosomes after stimulation with 100 ng/ml EGF (Catalog No. E9644). Preincubation with 1 µM Tyrphostin AG 1478 (Catalog No. T4182), a selective EGFR inhibitor, for 20 minutes prior to the addition of EGF blocks the internalization of the fusion protein.

Cell Line Description
Organism*: Homo sapiens* (human)

Tissue: Carcinoma; Lung

Age: 58 years

Gender: Male

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X, Y
CSF1PO: 10, 12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8, 11
TH01: 8,9.3
TPOX: 8, 11
vWA: 14

Parental Cell Line: ATCC® Catalog No. CCL-185™

Note: Please see CCL-185 product datasheet from ATCC for additional information about the origin of these cell lines. Cytogenetic information is based on initial seed stock at Sigma Life Science. Cytogenetic instability has been reported in the literature for some cell lines.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Biosafety Level: 1
This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. The parental cell line, A549, was obtained from ATCC. All animal products used in the preparation of the knockout line and maintenance of both, parental and knockout clone, have been screened negative by 9CFR for adventitious viral agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. Appropriate safety procedures are recommended to be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures have been published.[2-4]

Storage/Stability
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.

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 submersed 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.

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.

Complete Medium Preparation Instructions
To make the complete growth medium, add L-Glutamine, Catalog No. G7513, at a final concentration of 2 mM, and fetal bovine serum, Catalog No. F2442, to a final concentration of 10% in the base medium, RPMI-1640 Medium, Catalog No. R0883. This medium is formulated for use with a 5% CO_2 in air atmosphere.

Procedure
Thawing of Frozen Cells.
1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (~2 minutes).
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. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL of Complete Medium and spin at ~125 × g for 5–7 minutes.
4. Resuspend cell pellet with the Complete Medium and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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).
5. Incubate the culture at 37 °C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended for the Complete Medium.

Sub-culturing Procedure
Volumes used in this 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 Trypsin-EDTA solution (Catalog No. T3924)
3. Add 2.0–3.0 mL of Trypsin-EDTA solution to flask and incubate at 37 °C for 7 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: 1:3 to 1:20
6. Incubate cultures at 37 °C.

Note: More information on enzymatic dissociation and subculturing of cell lines is available in the literature.[4]

References

1. Ono, M. & Kuwano, M., Molecular mechanisms of epidermal growth factor receptor (EGFR) activation and response to gefitinib and other EGFR-targeting drugs. *Clin. Cancer Res*., **12**, 7242-7251 (2006).
2. Lurje, G. & Lenz, H.J., EGFR signaling and drug discovery. Oncology, **77**, 400-410 (2009).
3. Sorkin, A. & Goh, L.K., Endocytosis and intracellular trafficking of ErbBs. *Exp. Cell Res*., **315**, 683-696 (2009).
4. Centers for Disease Control, Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 21-1112. U.S. Dept. of Health and Human Services; 5th Edition (2009) U.S. Government Printing Office Washington D.C. The entire text is available online at www.cdc.gov/biosafety/publications/index.htm
5. Fleming, D.O. & Hunt, D.L., Biological Safety: Principles And Practices, 4th Edition, ASM Press, Washington, DC (2006).
6. Freshney, R.I., Culture of Animal Cells, a manual of Basic Technique, 6th edition, published by John Wiley & Sons, Hoboken, NJ (2010).

Additional product and technical information can be obtained from the catalog references and the Sigma Life Science Website (www.wherebiobegins.com/biocells).

Please see the enclosed Label License Agreement (LLA) for further details regarding the use of this product. The LLA is also available on our website at www.wherebiobegins.com/biocells

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