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Product Information

A549 GFP-STAT1
Lung Carcinoma Cell Line with GFP-tagged STAT1

Catalog Number **CLL1158**
Storage Temperature –196 °C (liquid nitrogen)

TECHNICAL BULLETIN

Product Description
This product is a human A549 cell line in which the genomic STAT1 gene has been endogenously tagged with a Green Fluorescent Protein (GFP) gene using CompoZr® zinc finger nuclease (ZFN) technology. The cell line shows redistribution of STAT1 from the cytoplasm to the nucleus upon activation with a ligand such as interferon-gamma (INF-γ), making it useful for high content screening of compounds that activate STAT1 to find novel anti-cancer drugs.

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 used to insert a transgene for GFP into a desired target location – in front of the stop codon of the STAT1 locus – resulting in the target protein with its C-terminus fused to the N-terminus of GFP. A donor construct containing a fluorescent reporter gene (GFP) flanked by sequences homologous to the regions on either side of the genomic target site was nucleofected into the A549 cells along with ZFNs designed to cut near the genomic target site (see Figures 1a and 1b). Integration resulted in endogenous expression of the fluorescent fusion protein STAT1-GFP.

Knockin cells were sorted into single cells by flow cytometry and then expanded into clonal populations. Testing of these clones was used to select a single STAT1-GFP clone as a stable cell line (Figures 2 and 3). Junction PCR showed at least one allele is tagged (Figure 4a) and Southern analysis showed no off-target insertions of the GFP (Figure 4b).

STAT1 is a member of the Signal Transducers and Activators of Transcription family of transcription factors (e.g., Stats 1, 3, 4, 5, and 6) and is involved in the induction of downstream genes upon a stimulatory signal from either type I, II, or III interferons. In response to type II IFN (INF-γ), STAT1 becomes both tyrosine and serine-phosphorylated. The protein then forms a homodimer termed IFN-γ-activated factor (GAF), migrates into the nucleus, and binds to the IFN-γ-activated sequence (GAS) to drive the expression of target genes, inducing a cellular antiviral state. Thus, this protein is a very important player in the cellular antiviral response.

STAT1 is also deficient or inactive in many types of human tumors.[1-3] One study clearly demonstrated STAT1 expressed by tumor cells is a negative regulator of tumor angiogenesis and, hence, tumor growth and metastasis.[4] Some anticancer drugs may activate STAT1 and contribute to suppression of tumor growth. It has been shown that doxorubicin can potentiate STAT1 activation in response to IFN-γ, and that this combination results in enhanced apoptosis in breast cancer cells.[5] From this perspective, A549 cells expressing endogenously tagged STAT1 could serve as a valuable screening model to find STAT1 activators that will help in treating both viral infections and certain types of human cancer.

Numerous studies demonstrate STAT1 suppresses tumor formation. However, two members of the Stat family (STAT3 and STAT5) have been implicated in tumorigenesis in mice and humans, and they are considered to be powerful proto-oncogenes. The quest for effective and specific STAT3 inhibitors (as potential anticancer drugs) includes one important condition: this inhibitor should not simultaneously impair STAT1 activation in the cells, i.e., while cytokine-induced STAT3 nuclear translocation must be completely suppressed by the compound, INF-induced STAT1 translocation can not be impaired.

Taking this into account, we developed RFP-STAT3 (Catalog No. CLL1140) and STAT1-GFP A549 cell lines that could be used to identify effective and specific inhibitors of STAT3 by screening compound libraries in a high-content mode.

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

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

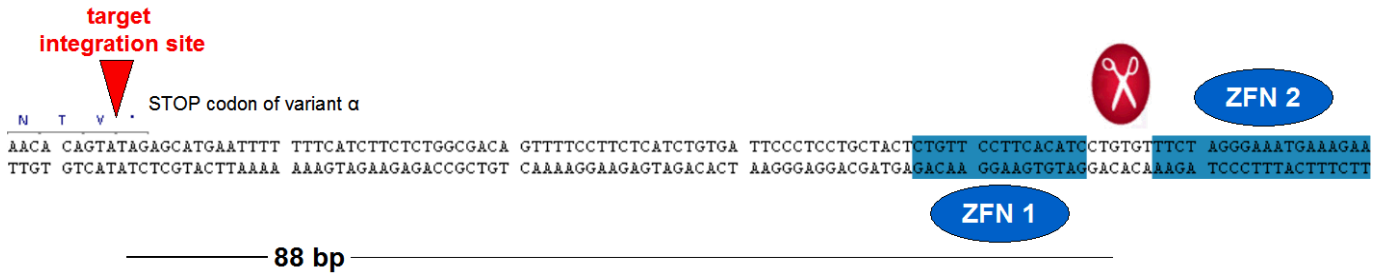
Component

A549 knockin cell line having the STAT1 gene tagged at the C-terminus with GFP Catalog No. CLL1167 — 1 vial

One vial of modified A549 cells contains ~2×10^6 cells in Cell Freezing Medium-DMSO 1×, Catalog No. C6164.

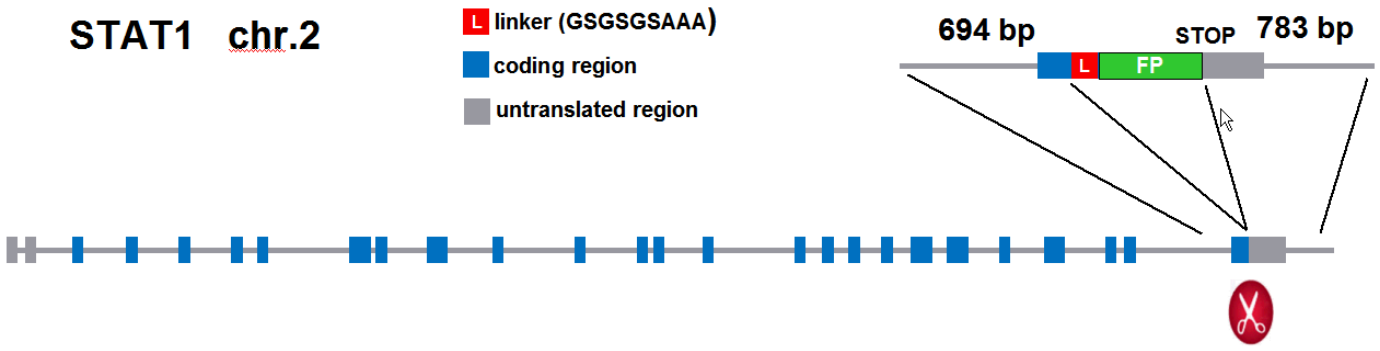
Design of tag sequence integration at the STAT1 gene locus

Figure 1a.



Schematic of the genomic sequence at the target region for integration of the fluorescent tag GFP. DNA of STAT1, showing the end of the last exon, CompoZr ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow).

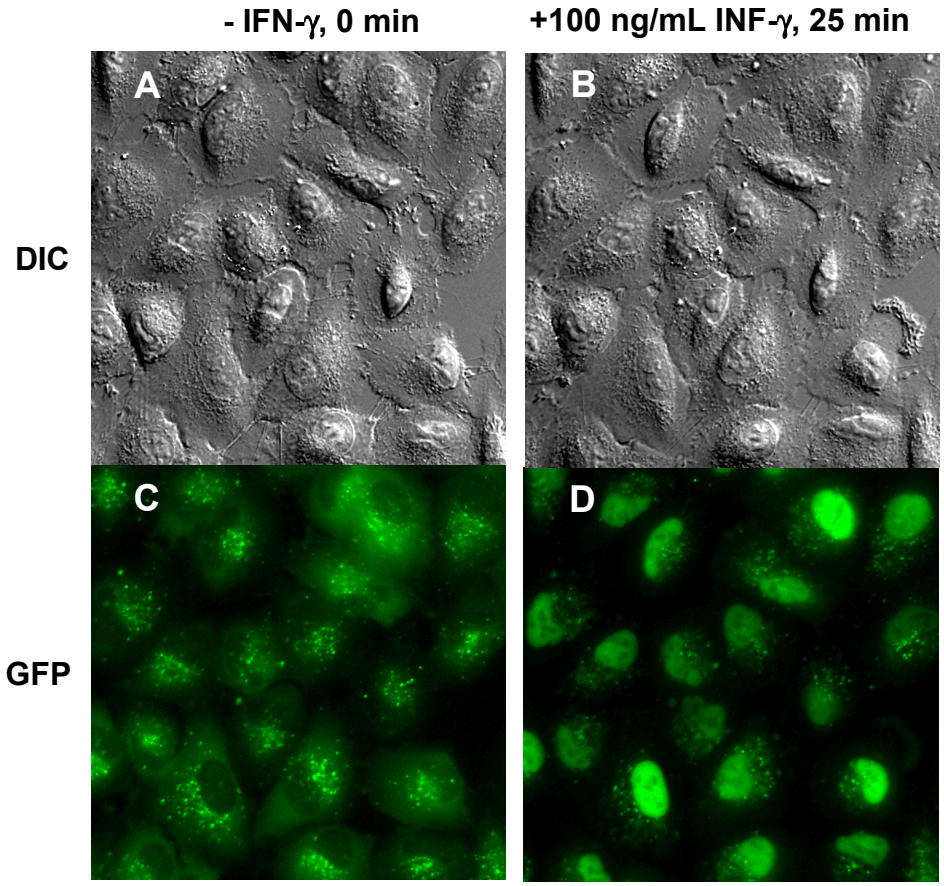
Figure 1b.



Schematic of the STAT1 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 to the end of the STAT1 coding sequence (a C-terminal fusion).

Localization and expression levels of endogenously tagged STAT1 (STAT1-GFP) in A549 cells

Figure 2.

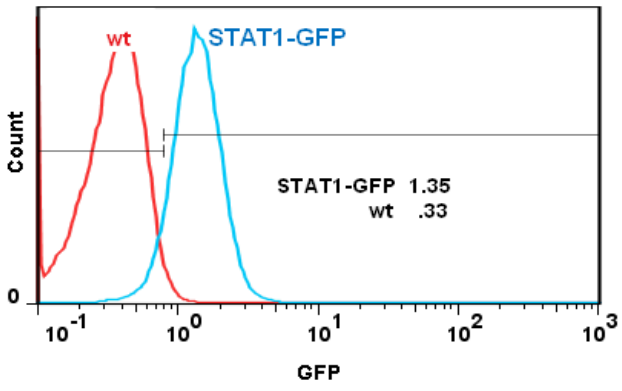


Differential interference contrast (DIC) and fluorescence microscopy images of an isolated single cell clone expressing the STAT1 gene (Signal Transducer and Activator of Transcription 1) endogenously tagged with GFP (A549 lung carcinoma) at the C-terminus. The images were taken either before (A, C) or 25 minutes after addition of 100 ng/mL IFN-γ (B, D). The cells were imaged live in Hanks balanced salt solution (Catalog No. H8264) supplemented with 2% fetal bovine serum (Catalog No. F2442) using a GFP filter set (ex 450–490/em 550) and 40x/1.3 oil objective. Endogenous STAT1 expression levels are low and near autofluorescence levels (see Figure 3). IFN-γ induced nuclear translocation of the STAT1-GFP fusion protein is readily detectable.

Expression levels of endogenously tagged STAT1 (STAT1-GFP) in A549 cells

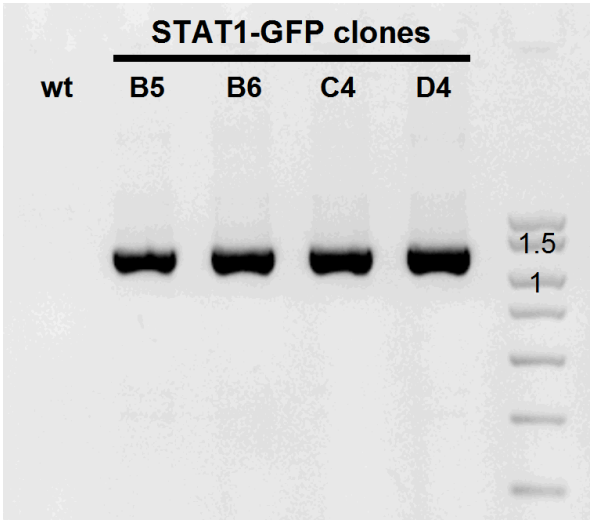
Figure 3.

Fluorescence analysis of the STAT1-GFP clone compared to the wild type A549 (autofluorescence) using MACSQuant®.
The figures on the right are fluorescent mean intensities.



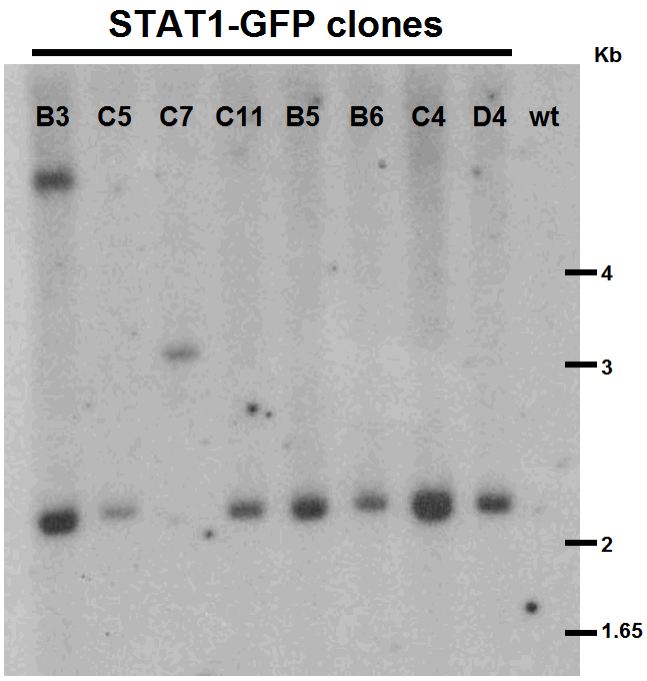
Molecular analysis to identify targeted integration in A549 STAT1-GFP clones

Figure 4a.



Junction PCR was performed on genomic DNAs isolated from the wild type (wt) and STAT1-GFP clones. 1.2 kb expected fragment for left junction confirms the targeted integration of GFP in front of the stop codon of the STAT1 locus for all clones tested. No PCR product can be detected in the wild type control. Junction PCR products were confirmed by sequencing.

Figure 4b.



Southern blotting tests for possible random integration of the plasmid donor initially used to create the clones. Genomic DNA from single cell clones and from A549 wild type (wt - served as a negative control) were digested with *Nde* I restriction endonuclease. Proper targeted insertion of the GFP into the STAT1 locus should produce a hybridized band of ~2.1 kb in size. Radioactive-labeled GFP probe was used. Clone C4 was chosen as the final product based on cell morphology, molecular analyses and imaging/translocation analyses.

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

The STR profile of this cell line matches that of its 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.[6-8]

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.[8]

References

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4. Huang, S. et al., Stat1 negatively regulates angiogenesis, tumorigenicity and metastasis of tumor cells. Oncogene, **21**(16), 2504-12 (2002).
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Additional product and technical information can be obtained from the catalog references and the Sigma Life Science Website (www.wherebiobegins.com/biocells).

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