

Product Information

CompoZr® Disease Model Cell Lines A549 Cells SMAD4 -/-

Catalog Number **CLLS1013**

Storage Temperature -196 °C (liquid nitrogen)

Product Description

CompoZr® zinc finger nuclease (ZFN) 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 non-homologous end joining pathway resulted in deletions at the SMAD4 locus (see Figures 1a and 1b). Single cell knockout clones were isolated and followed for more than twenty passages to establish stable cell lines.

While the targeted gene in this cell line is diploid, ZFN-mediated gene knockout technology is not limited to diploid targets, allowing the researcher to pursue many of the polyploid cell lines often characteristic of cancer. Modified cell lines provide the basis for the development of various assays for compound screening. Here, the target gene and corresponding protein expression are eliminated, in contrast to cell lines with normal expression.

Loss or reduction of expression of mother against decapentaplegic homologue 4 (SMAD4) has been associated with potential onset of lung cancer as well as colorectal cancer.¹⁻³ The product of the SMAD4 gene is generally regarded as the signaling mediator of the TGF-β pathway and suggested to act as a tumor suppressor.⁴ Generation of this cell line allows the examination of antitumor compounds in the absence of SMAD4, as well as the investigation of the possible roles of other relevant pathway components.

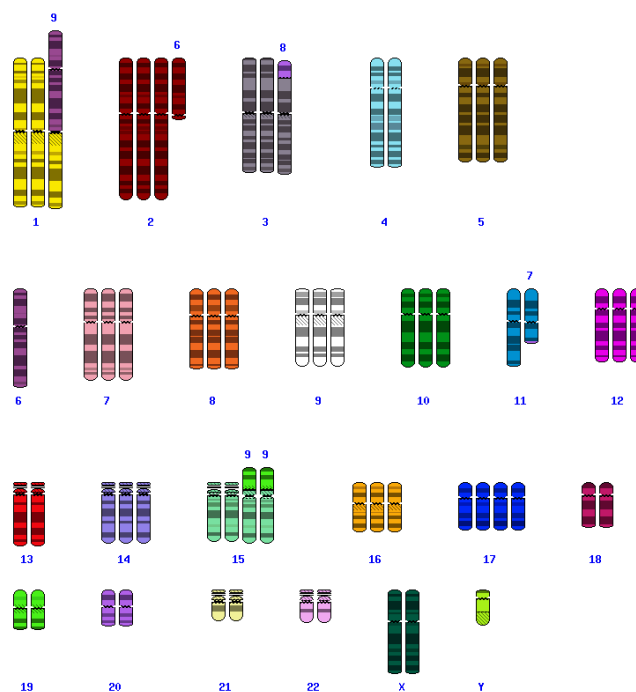
For further information and to download sequence of modified locus, go to the website:
www.wherebiobegins.com/biocells

Components

A549 mutant cell line with SMAD4 gene knocked out 1 vial
 Catalog No. CLL1018

Parental colon adenocarcinoma cell line 1 vial
 (ATCC® Catalog No. CCL-185™)
 Catalog No. CLL1015

Figure 1a.
 Creation of SMAD4 Knockout in A549 Cells



SMAD4 is diploid in A549 cell line: location – 18q21.1
 (from NCBI SKY/M-FISH database)

Figure 1b.

Site-specific deletion at the SMAD4 Locus in the A549 cell line

Allele 1 – 8 bp + 6 bp deletion:

CTGATAGGCCATGGGTGAGTTACACTTTTTGCCCA
TCTTTATAGTTGTGCATTATCAGATAAAATTGGTCCT
TCATTTAGTATATGAAATCATAAGATGACATCTATGA
ATGTACCATGTTAATGTCTTCTTGTTCCTCTAGGTC
AGCCTGCCAGTATACTGGGGGGCAGCCATAGTGA
AGGACTGTTTCAGATAGCATCA**GGGCCTCAGCCAG**
GACAGCagcaga**ATGGATTTACTGGTCAGCC**AGCTA
CTTACCATCATAGTATGTACATACTTTAAAAAATCTT
TTAAATAGTTGAGAAAAAAGTAGGCAGCCTTTATAA
AAGCAAATTAACCCATGTGGGCCTTAATTTTTAGAC
AGCACTACCACCTGGACTGG

Allele 2 – 8 bp + 15 bp deletion:

CTGATAGGCCATGGGTGAGTTACACTTTTTGCCCA
TCTTTATAGTTGTGCATTATCAGATAAAATTGGTCCT
TCATTTAGTATATGAAATCATAAGATGACATCTATGA
ATGTACCATGTTAATGTCTTCTTGTTCCTCTAGGTC
AGCCTGCCAGTATACTGGGGGGCAGCCATAGTGA
AGGACTGTTGCAGATAGCATCAGGGC**CTCAGCCA**
GGACAGCagcaga**ATGGATTTACTGGTCAGCC**AGCT
ACTTACCATCATAGTATGTACATACTTTAAAAAATCT
TTAAATAGTTGAGAAAAAAGTAGGCAGCCTTTATA
AAAGCAAATTAACCCATGTGGGCCTTAATTTTTAGA
CAGCACTACCACCTGGACTGG

Schematic of the genomic sequence at the target region recognized by the ZFN pair flanking the ZFN cut site, the resulting deletion and the CEL-I primer sequences:

Cel 1 Primers - **Bolded and underlined**
ZFN binding site - **UPPER CASE, BOLDED RED**
ZFN cut site - **lower case red**
Deletion - **yellow highlighted**

Genotype: del 8+6/del 8+15 (heterozygous)

Cell Line Description

1 vial of modified A549 cells contains $\sim 2 \times 10^6$ cells.

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.

Medium: Fetal bovine serum, Catalog No. F4135, at a final concentration of 10% (v/v) in F-12 Hams Nutrient Mixture, Catalog No. N4888, supplemented with L-glutamine (Catalog No. G7513) to a final concentration of 2 mM. This medium is formulated for use with a 5% CO₂ in air atmosphere.

The cryoprotectant medium used is 1× Cell Freezing Medium-DMSO, Catalog No. C6164.

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.⁵⁻⁸

Preparation Instructions

Complete Medium: To make the complete growth medium, add fetal bovine serum, Catalog No. F4135, to a final concentration of 10% (v/v) in the base medium, F-12 Ham, Catalog No. N4888. The medium is supplemented with L-glutamine, Catalog G7513, to a final concentration of 2 mM. This medium is formulated for use with a 5% CO₂ in air.

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.

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 cm² or a 75 cm² 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% CO₂ in air atmosphere is recommended for the Complete Medium.

Subculturing Procedure

Volumes used in this procedure are for a 75 cm² flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

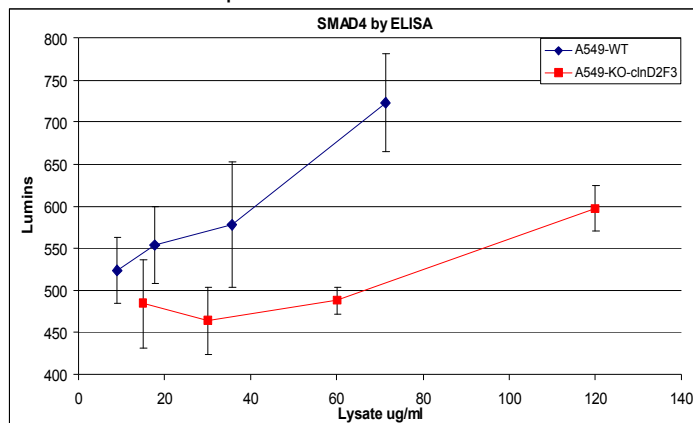
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 2.0–3.0 ml of Trypsin-EDTA solution to flask and incubate at 37 °C for 10 minutes to detach the cells.
4. Add 6.0–8.0 ml of Complete Medium and aspirate cells by gentle pipetting.
5. Add appropriate aliquots of the cell suspension into new culture vessels.
Subcultivation Ratio: 1:3 to 1:6
6. Incubate cultures at 37 °C.

Note: More information on enzymatic dissociation and subculturing of cell lines is available in the literature.⁹

Results

Figure 2.

Loss of SMAD4 expression



SMAD4 expression was examined in wild type A549 and the knockout cloned line using an enzyme-linked immunosorbent assay. Cell lysates were prepared using RIPA buffer as outlined in lysis procedure for Catalog No. R0278. Total protein concentration was determined by BCA assay (Catalog No. QPBCA). Briefly, 96-well plates were coated overnight at 2–8 °C with capture antibody (Catalog No. S3934, mouse monoclonal anti-SMAD4) diluted 1:1000 in carbonate/bicarbonate buffer (Catalog No. C3041). Plates were washed and blocked with a 1% (v/v) BSA solution. Serial dilutions of cell lysate prepared in 0.1% (v/v) BSA were added at 200 µl per well and incubated overnight at 2–8 °C. Plates were washed and the detection antibody (R&D Systems biotinylated goat anti-human SMAD4) was added and incubated 4 hours at room temperature. Plates were washed and incubated with streptavidin-peroxidase (R&D Systems 890803) for 2 hours at room temperature. The ELISA plates were developed using a chemiluminescent peroxidase substrate (Catalog No. CPS2120). Technical triplicates were examined for each concentration.

References

1. Takenaka, K. et al., Reduced transcription of the Smad4 gene during pulmonary carcinogenesis in idiopathic pulmonary fibrosis. *Molec. Med. Reports*, **2** (1), 73-80 (2009).
2. Salovaara, R. et al., Frequent loss of SMAD4/DPC4 protein in colorectal cancers. *Gut*, **51**, 56-59 (2002).
3. Papageorgis, P. et al., Smad4 inactivation promotes malignancy and drug resistance of colon cancer. *Cancer Res.*, **71**(3), 1-11 (2011).
4. Hahn, S.A. et al., DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. *Science*, **271**, 350-353 (1996).
5. Centers for Disease Control (1999), Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services; 4th Edition U.S. Government Printing Office Washington D.C. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
6. Fleming, D.O. et al., (1995) *Laboratory Safety: Principles and Practice*. Second edition, ASM press, Washington, DC.
7. Hay, R.J. et al., eds. (1992), *ATCC Quality Control Methods for Cell Lines*. 2nd edition, Published by ATCC.
8. Caputo, J.L., *Biosafety procedures in cell culture*. *J. Tissue Culture Methods*, **11**, 223-227 (1988).
9. Freshney, R.I., Chapter 10 in *Culture of Animal Cells, a manual of Basic Technique* by, 3rd edition, published by Alan R. Liss, (NY, NY: 1994).

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

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