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Product Information

CompoZr® Disease Model Cell Lines
SW48 Cells SMAD4 –/–

Catalog Number **CLLS1010**
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 most often observed as 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.

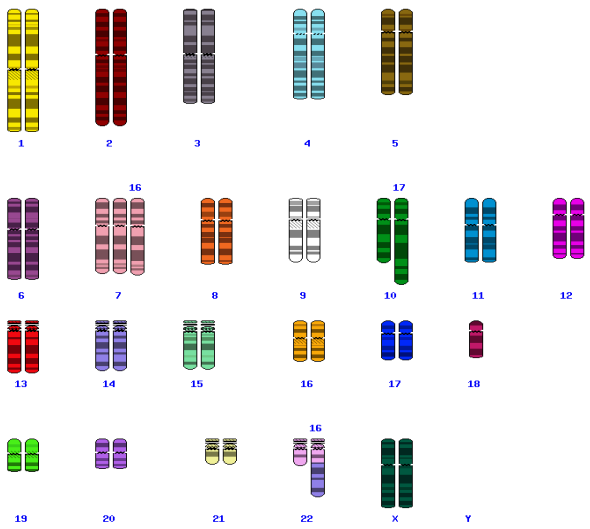
Loss or reduction of expression of mother against decapentaplegic homologue 4 (SMAD4) has been associated with colorectal cancer.[1,2] The product of the SMAD4 gene is generally regarded as the signaling mediator of the TGFb pathway and suggested to act as a tumor suppressor.[3] Generation of this cell line with 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

SW48 mutant cell line with SMAD4 gene knocked out Catalog No. CLL1012	1 vial
Parental colon adenocarcinoma cell line (ATCC® Catalog No. CCL-231™) Catalog No. CLL1008	1 vial

Figure 1a.
Creation of SMAD4 Knockout in SW48 Cells



SMAD4 is most often observed as diploid in SW48 cell line (17 of 20 cells examined): location – 18q21.1

Karyotype: 47,XX,+7[16],dup(10)(q21.3q23.1)[17],-18[3],der(22)t(14;22)(q12;qter)[16][cp17]
(from NCBI SKY/M-FISH database)

Figure 1b.
Site-specific deletion at the SMAD4 Locus in SW48 cell line

Alleles 1 and 2 – 67 bp deletion:

CTGATAGGCCATGGGTGAGTTACACTTTTTGCCCA
TCTTTATAGTTGTGCATTATCAGATAAAATTGGTCCT
TCATTTAGTATATGAAATCATAAGATGACATCTATGA
ATGTACCATGTTAATGTCTTCTTGTTCCTCTAGGTC
AGCCTGCCAGTATACTGGGGGGCAGCCATAGTGA
AGGACTGTTGCAGATAGCATCAGGGC**CTCAGCCA**
GGACAGCagcagaATGGATTTACTGGTCAGCCAGC
TACTTACCATCATAGTATGTACATACTTTAAAAAATC
TTTTAAATAGTTGAGAAAAAAGTAGGCAGCCTTTAT
AAAAGCAAATTAACCCATGTGGGCCTTAATTTTTAG
AC**AGCACTACCACCTGGACTGG**

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

CEL-I Primers - **Bolded and underlined**
ZFN binding site – **UPPER CASE, BOLDED RED**
ZFN cut site - **lower case, bolded red**
Deletion - yellow highlighted

Genotype: del 67/del 67: homologous deletion

SW48 Clone: A2

Cell Line Description
1 vial of modified SW48 cells contains ~2×10^6 cells.

Organism*: Homo sapiens* (human)

Tissue: adenocarcinoma; colorectal

Age: 82 years

Gender: Female

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X
CSF1PO: 9, 10
D13S317: 11, 12
D16S539: 11, 13
D5S818: 10, 14
D7S820: 9, 10
TH01: 6, 9.3
TPOX: 8
vWA: 18, 20, 21

Parental Cell Line: ATCC Catalog No. CCL-231
Note: Please see CCL-231 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 DMEM, Catalog No. D5671, supplemented with L-glutamine, Catalog No. G7513, to a final concentration of 2 mM and sodium pyruvate at 1 mM final concentration, Catalog No. S8636. This medium is formulated for use with a 5% CO_2 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, SW48, 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.[4-7]

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, DMEM, Catalog No. D5671. The medium is supplemented with L-glutamine, Catalog No. G7513, to a final concentration of 2 mM and sodium pyruvate, Catalog No. S8636, to a final concentration of 1 mM. This medium is formulated for use with a 5% CO_2 in air atmosphere.

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 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.

Subculturing 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 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.[8]

References

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2. Papageorgis, P. et al., Smad4 inactivation promotes malignancy and drug resistance of colon cancer. Cancer Res., **71(3)**, 1-11 (2011).
3. Hahn, S.A. et al., DPC4, a candidate tumor suppressor gene at human chromosome 18q21.1. Science, 271, 350-353 (1996).
4. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC.
5. Hay, R.J. et al., eds., ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC (1992).
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8. 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.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 Web site at www.wherebiobegins.com/biocells

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