3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

CompoZr® Disease Model Cell Lines
SW48 Cells TP53 –/–

Catalog Number **CLLS1007**
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 TP53 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.

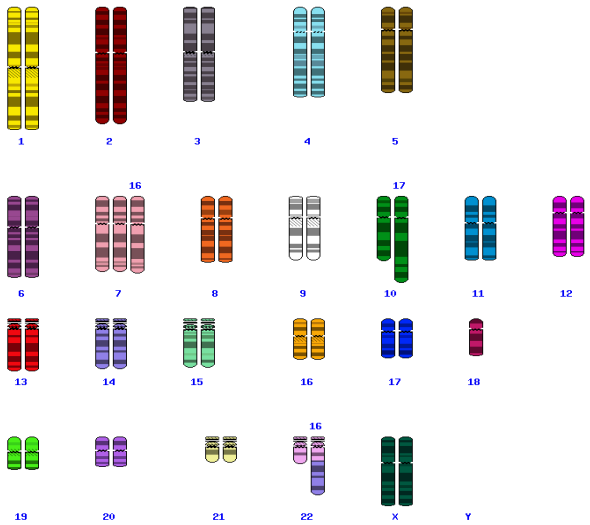
Tumor Protein 53 (TP53) was first recognized to be associated with cancer in 1979[1] and later determined to be a tumor suppressor.[2] TP53 has been well documented in its association with cancer and was deemed "molecule of the year" in 1993 by Science.[3] Additionally, TP53 mutation or loss has been widely associated with colorectal cancer,[1,4] but is shown to be expressed at the wild type level on the colorectal cell line SW48.[4]

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

Components

SW48 mutant cell line with TP53 gene knocked out Catalog No. CLL1013	1 vial
Parental colon adenocarcinoma cell line (ATCC Catalog No. CCL-231) Catalog No. CLL1008	1 vial

Figure 1a.
Creation of TP53 Knockout in SW48 Cells



TP53 is diploid in SW48 cell line: location – 17p13.1 (from NCBI SKY/M-FISH database)

Figure 1b.
Site-specific deletion at the TP53 Locus in SW48 cell line – Alleles 1 and 2:

GATGTGATGAGAGGTGGATGGGTAGTAGTATGGA
AGAAATCGGTAAGAGGTGGGCCCAGGGGTCAGAG
GCAAGCAGAGGCTGGGGCACAGCAGGCCAGTGTG
CAGGGTGGCAAGTGGCTCCTGA**CCTGGAGTCTTC**
CAGTGTgatgat**GGTGAGGATGGGCCTCCG**GTTCAT
CCGCCCATGCAGGAACTGTTACACATGTAGTTGTA
GGGATGGTGGTACAGTCAGAGCCAACCTAGGAGA
TAACACAGG**CCCAAGATGAGGCCAGTGCGCCTTG**

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 red
Deletion – yellow highlighted

Genotype: del 11/ del 11 (homozygous)

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, Catalog No. S8636, at 1 mM final concentration. 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.[5-7]

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 vessel exploding or 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]

Results

Figure 2.
Loss of TP53 expression

TP53 expression was examined by Western blot analysis for the wild type SW48 cell line and the TP53 knockout clone. Lanes 1 & 4 contain wild type nuclear lysate; lanes 2 & 6 contain nuclear lysate from the knockout line; lanes 3 & 5 contain cytoplasmic lysate from knockout or wild type lines, respectively. Briefly, both cell populations were treated with complete media containing 5 μM 5-FU (5-fluorouracil, Catalog No. F6627) 24 hours prior to harvesting cytoplasmic and nuclear protein fractions. A total of 18 μg (lanes 1 & 6) or 36 μg (lanes 2-5) of protein, determined by BCA assay (Catalog No. QPBCA), were loaded on a 4-20% tris-glycine gel (Invitrogen EC60249). After transferring to PVDF, the membrane was blocked with 3% w/v nonfat dry milk (Catalog No. M7409), probed with primary monoclonal anti-TP53 antibody (Catalog No. P6749), followed by the peroxidase-conjugated secondary antibody (Catalog No. A0168). Signals were developed by incubating with the peroxidase substrate TMB (Catalog No. T0565).

References

1. Lane, D.P., and Crawford, L.V., T antigen is bound to a host protein in SV40-transformed cells. Nature, **278**, 261-263 (1979).
2. Baker, S.J. et al., Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science, **244**(4901), 217-221 (1989).
3. Koshland, D.E., Molecule of the year. Science, **262**(5142), 1953 (1993).
4. Liu, Y., and Bodmer, W.F., Analysis of P53 mutations and their expression in 56 colorectal cancer lines. Proc. Natl. Acad. Sci., **103**(4), 976-981 (2006).
5. Hay, R.J. et al., eds., ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC (1992).
6. Caputo, J.L., Biosafety procedures in cell culture. J. Tissue Culture Methods, **11**, 223-227 (1988).
7. 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
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 website at www.wherebiobegins.com/biocells

These cells are distributed for research purposes only. Sigma Life Science requires that individuals contemplating commercial use of any cell line first contact us to negotiate an agreement. Third party distribution of this cell line is prohibited.

AS,MAM 01/11-1