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Product Information

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

Catalog Number **CLLS1009**
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 PTEN 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.

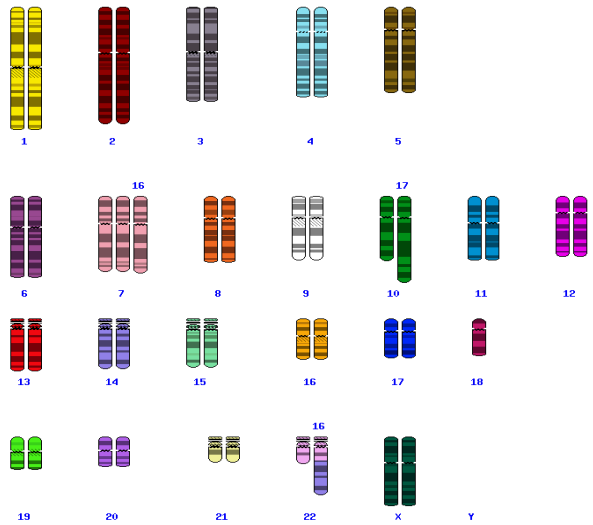
The gene encoding phosphatase and tensin homolog (PTEN) is known to act as a tumor suppressor.[1] This protein functions as a tumor suppressor by serving to help control the cell cycle, regulating cellular division and acting as a negative regulator of the AKT/PKB signaling pathway.[2,3] Loss or reduction of PTEN expression has been shown to be strongly associated with primary sporadic colorectal cancer.[4] The availability of cell lines with PTEN expression knocked out allows the investigation of interactive pathways and potential approaches of therapeutic agents.[5]

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

Components

SW48 mutant cell line with PTEN gene knocked out Catalog No. CLL1011	1 vial
Parental colon adenocarcinoma cell line (ATCC Catalog No. CCL-231) Catalog No. CLL1008	1 vial

Figure 1a.
Creation of PTEN Knockout in SW48 Cells



PTEN is diploid in SW48 cell line: location – 10q23.3 (from NCBI SKY/M-FISH database)

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

Allele 1:
CCTGTTAAGTTTGTATGCAACATTTCTAAAGTTACC
TACTTGTTAATTAAAAATTCAAGAGTTTTTTTTTCTTA
TTCTGAGGTTATCTTTTTACCACAGTTGCACAATAT
CCTTTTGAAGACCATAACCCACCACAGCTAGAA**CTT**
ATCA**AACCCT**tttgt**GAAGAT**CTT**GACCAATGG**CTAA
GTGAAGATGACAATCATGTTGCAGCAATTCACTGTA
AAGCTGGAAAGGGACGAACTGGTGTAATGATATGT
GCATATTTATTACATCGGGGCAAATTTT**TAAAGGCA**
CAAGAGGCCCTA

Allele 2:
CCTGTTAAGTTTGTATGCAACATTTCTAAAGTTACC
TACTTGTTAATTAAAAATTCAAGAGTTTTTTTTTCTTA
TTCTGAGGTTATCTTTTTACCACAGTTGCACAATAT
CCTTTTGAAGACCATAACCCACCACAGCTAGAA**CTT**
ATCA**AACCCT**tttgt**GAAGAT**CTT**GACCAATGG**CTAA
GTGAAGATGACAATCATGTTGCAGCAATTCACTGTA
AAGCTGGAAAGGGACGAACTGGTGTAATGATATGT
GCATATTTATTACATCGGGGCAAATTTT**TAAAGGCA**
CAAGAGGCCCTA

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: 1 del* + 103 del / 1 del* + 24 del

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.[6-8]

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, 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.

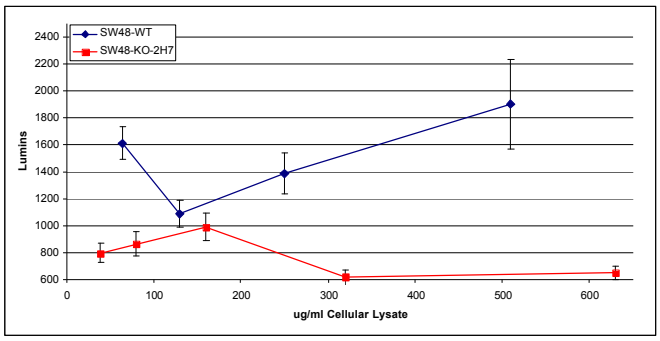
Subculturing Procedure
Volumes used in this procedure are for a 75 cm^2 flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 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.[9]

Results

Figure 2.

Loss of PTEN expression



PTEN expression was examined in wild type SW48 and the knockout cloned line using an enzyme-linked immunosorbent assay (a modification of R&D Systems DYC847-2). Briefly, both cell populations were grown to near confluency in T75 flasks prior to harvesting as outlined in the "Subculturing Prodecure". Cytoplasmic cellular lysates were prepared as outlined in the procedure (Pierce, NE-PER 78833). Total protein concentration was determined by BCA assay (Catalog No. QPBCA). Technical triplicates were examined for each concentration. The ELISA plates were developed using a chemiluminescent peroxidase substrate (Catalog No. CPS260).

References

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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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