

Product Information

CompoZr® Disease Model Cell Lines DLD-1 Cells PTEN -/-

Catalog Number **CLLS1004**

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 insertions 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 The 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

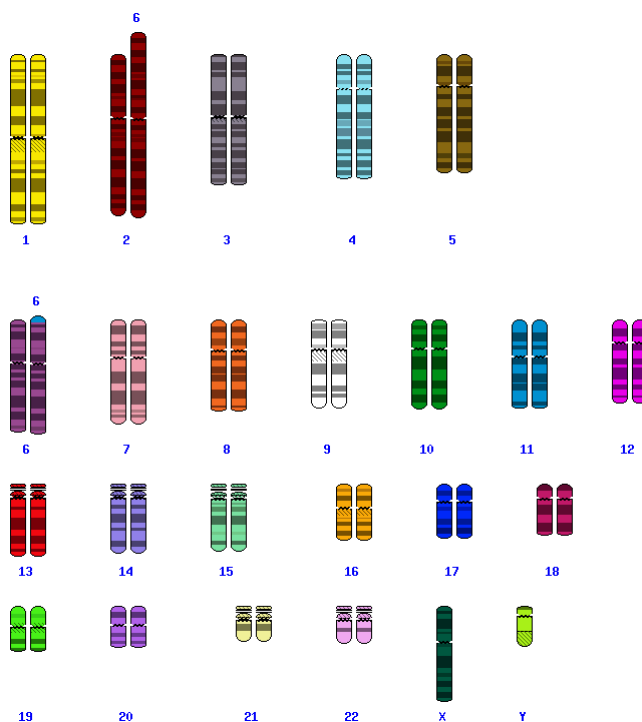
For further information and to download sequence of modified locus, go to the website:
www.wheribiobegins.com/biocells

Components

DLD-1 mutant cell line with PTEN gene knocked out 1 vial
 Catalog No. CLL1005

Parental colon adenocarcinoma cell line (ATCC Catalog No. CCL-221) 1 vial
 Catalog No. CLL0001

Figure 1a.
 Creation of PTEN Knockout in DLD-1 Cells



PTEN is diploid in DLD-1 cell line: location – 10q23.3 (from NCBI SKY/M-FISH database)

Figure 1b.

Site-specific insertion at the PTEN Locus in DLD-1 cell line

Allele 1:

GATACAGAATCCATATTTGCGTGTATATTGCTG
 ATATTAATCATTAAAATCGTTTTTGACAGTTTGA
 CAGTTAAAGGCATTTCTGTGAAATAATACTG
 GTATGTATTTAACCATGCAGATCCTCAGTTTGT
 GGTCTGCCAGCTAAAGGTGAAGATATATTCCT
 CCAATT**CAGGACCCACACgacg**TGATCACGGC
ACTGCACTCCAGCCTGGGCAACATAGCAAAAC
CCTGGGTGGGAAGGAAGGAAGGGAGGAAGG
GAGGAAGGGAGGAAGGGAGGGCGGGAAAGG
AAGAAAGAAGGCATGAGTTTTATATTGAGGGG
AAGACGgGAAGACAAGTTCATGTACTTTGAGT
 TCCCTCAGCCGTTACCTGTGTGTGGTGATATC
 AAAGTAGAGTTCTTCCACAAACAGAACAAGAT
 GCTAAAAAAGGTTTGTACTTTACTTTTATTGGG
 AGAAATATCCAAAATAAGGACAGATTAAGGCT
 ATATTTTATTTTATGACATGTAAGGAAGTATAAT
 TTGTTTTCT**TATTAGATCTGCAGGTGTTTTGCTT**
ACTCT

Allele 2:

GATACAGAATCCATATTTGCGTGTATATTGCTG
 ATATTAATCATTAAAATCGTTTTTGACAGTTTGA
 CAGTTAAAGGCATTTCTGTGAAATAATACTG
 GTATGTATTTAACCATGCAGATCCTCAGTTTGT
 GGTCTGCCAGCTAAAGGTGAAGATATATTCCT
 CCAATT**CAGGACCCACACgacg**GACGg**GAAGA**
CAAGTTCATGTACTTTGAGTTCCTCAGCCGT
 TACCTGTGTGTGGTGATATCAAAGTAGAGTTC
 TTCCACAAACAGAACAAGATGCTAAAAAAGGT
 TTGTACTTTACTTTTATTGGGAGAAATATCCAA
 AATAAGGACAGATTAAGGCTATATTTTATTTT
 ATGACATGTAAGGAAGTATAATTTGTTTTCT**AT**
TAGATCTGCAGGTGTTTTGCTTACTCT

Schematic of the genomic sequence at the target region (exon 7) 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**

Insertion – **yellow highlighted**

Exon 7 – UPPER CASE (except for cut site)

Genotype: ins 140/ ins 4

Cell Line Description

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

Organism: *Homo sapiens* (human)

Tissue: adenocarcinoma; colorectal

Age: adult

Gender: Male

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile

Short Tandem Repeat (STR) analysis:

Amelogenin: X,y

CSF1PO: 11, 12

D13S317: 8, 11

D16S539: 12,13

D5S818: 13

D7S820: 10, 12

TH01: 7, 9.3

TPOX: 8, 11

vWA: 18, 19

Parental Cell Line: ATCC Catalog No. CCL-221

Note: Please see CCL-221 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 RPMI, Catalog No. R5886, 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% 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, DLD-1, 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, RPMI, Catalog No. R5886. 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% CO₂ 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 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.

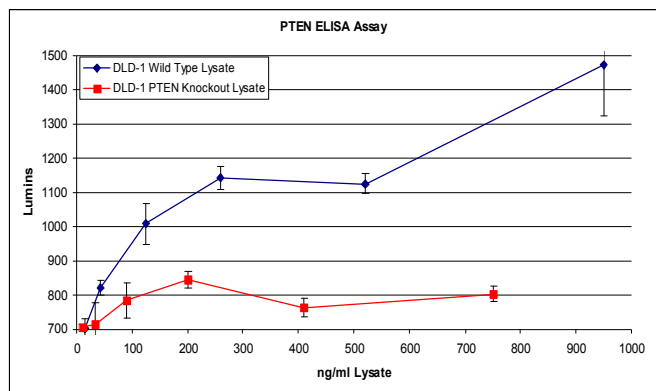
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 2.0–3.0 ml of Trypsin-EDTA solution to flask and incubate at 37 °C for 10 minutes to detach the cells.
4. Add 6.0–8.0 ml of Complete Medium and aspirate cells by gentle pipetting.
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 PTEN expression



PTEN expression was examined in wild type DLD-1 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 Procedure". 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

1. Steck, P.A. et al., Identification of a candidate tumor suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat. Genet.*, **15**(4), 356-362 (1997).
2. Chew, E.C., and Tarnawski, A.S., PTEN regulatory functions in tumor suppression and cell biology. *Med. Sci. Monit.*, **10**(10), RA235-241 (2004).
3. Stambolic, V. et al., Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*, **95**, 29-39 (1998).
4. Nassif, N.T. et al., PTEN mutations are common in sporadic microsatellite stable colorectal cancer. *Oncogene*, **23**, 617-628 (2004).
5. Saito, Y. et al., Adenovirus-mediated PTEN treatment combined with caffeine produces a synergistic therapeutic effect in colorectal cancer cells. *Cancer Gene Ther.*, **10**(11), 803-813 (2003).
6. Hay, R.J. et al., eds., *ATCC Quality Control Methods for Cell Lines*. 2nd edition, Published by ATCC (1992).
7. Caputo, J.L., *Biosafety procedures in cell culture*. *J. Tissue Culture Methods*, **11**, 223-227 (1988).
8. 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
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.wherebiobegins.com/biocells).

CompoZr is a registered trademark of Sigma-Aldrich® Biotechnology LP and Sigma-Aldrich Co.

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

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.