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
MCF10A Cells PTEN –/–

Catalog Number **CLLS1046**
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 Figure 1). 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 is disrupted, in contrast to cell lines with normal expression (see Figure 2).

The PTEN (Phosphatase and Tensin Homolog) gene was identified as a tumor suppressor mutated in a large number of cancers at high frequency.[1,2] This gene encodes a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase which acts as a dual-specificity protein phosphatase and lipid phosphatase. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and antagonizes the PI3K-AKT/PKB signaling pathway by dephosphorylating phosphoinositides, thereby, modulating cell cycle progression and cell survival.[3]

PTEN is one of the most commonly lost tumor suppressors in human cancer. During tumor development, mutations and deletions of PTEN occur that inactivate its enzymatic activity leading to increased cell proliferation and reduced cell death. Frequent genetic inactivation of PTEN occurs in glioblastoma, endometrial cancer, prostate cancer, and reduced expression is found in many other tumor types including lung and breast cancer.[3-6]

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

Figure 1.
Creation of PTEN Knockout in MCF10A Cells

Site-specific deletion at the PTEN Locus in MCF10A cell line.

Allele 1 – 19 bp deletion:

TTTAAATA**CCTGTTAAGTTTGTATGCAACAT**TTCTA
AAGTTACCTACTTGTTAATTAAAAATTCAAGAGTTTT
TTTTTCTTATTCTGAGGTTATCTTTTTACCACAGTTG
CACAATATCCTTTTGAAGACCATAACCCACCACAGC
TAGAA**CTTATCAAACCCT**tttgt**GAAGATC**TTGACCAA
TGGCTAAGTGAAGATGACAATCATGTTGCAGCAATT
CACTGTAAAGCTGGAAAGGGACGAACTGGTGTAAT
GATATGTGCATATTTATTACATCGGGGCAAATTTT**T**
AAAGGCACAAGAGGCCCTAGATTTCTA

Allele 2 – 2 bp deletion:

TTTAAATA**CCTGTTAAGTTTGTATGCAACAT**TTCTA
AAGTTACCTACTTGTTAATTAAAAATTCAAGAGTTTT
TTTTTCTTATTCTGAGGTTATCTTTTTACCACAGTTG
CACAATATCCTTTTGAAGACCATAACCCACCACAGC
TAGAA**CTTATCAAACCCT**tttgt**GAAGATCTTGACCAA**
TGGCTAAGTGAAGATGACAATCATGTTGCAGCAATT
CACTGTAAAGCTGGAAAGGGACGAACTGGTGTAAT
GATATGTGCATATTTATTACATCGGGGCAAATTTT**T**
AAAGGCACAAGAGGCCCTAGATTTCTA

Schematic of the genomic sequence at the target region (exon 11) 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 19/del 2 (heterozygous)

Components

MCF10A mutant cell line with PTEN gene knocked out — 1 vial
Catalog Number CLL1046

Parental mammary epithelial cell line (ATCC® Catalog Number CRL-10317™) — 1 vial
Catalog Number CLL1040

1 vial of modified MCF10a cells contains ~2×10^6 cells.

The cryoprotectant medium used is 1× Cell Freezing Medium-DMSO, Catalog Number C6164.

Cell Line Description
Organism*: Homo sapiens* (human)

Tissue: mammary gland; breast

Age: 36 years

Gender: Female

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X
CSF1PO: 10,12
D13S317: 8,9
D16S539: 11,12
D5S818: 10,13
D7S820: 10,11
THO1: 8,9.3
TPOX: 9,11
vWA: 15,17

Parental Cell Line: ATCC Catalog Number CRL-10317
Note: Please see CRL-10317 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.

Complete Medium: Dulbecco's Modified Eagle's Medium (DMEM)/Ham's Nutrient Mixture F12 (1:1) with 2.5 mM L-glutamine, 5% horse serum, 10 µg/mL human insulin, 0.5 µg/mL hydrocortisone, 10 ng/mL EGF, and 100 ng/mL cholera toxin. This medium is formulated for use with a 5% CO_2 in air atmosphere.

Medium Components:
Cholera Toxin from *V. cholerae*,
Catalog Number C8052
DMEM/F12, Catalog Number 51448C
Insulin Solution, Catalog Number I9278
Epidermal Growth Factor, Catalog Number E9644
50 µM Hydrocortisone Solution,
Catalog Number H6909
Horse Serum, Catalog Number H1270

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, MCF10A, 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[7-10].

Preparation Instructions

Complete Medium: To make the complete growth medium combine the following:

1 L of DMEM/F12 (Catalog Number 51448C)
50 mL of horse serum (Catalog Number H1270)
29 mL of 50 µM Hydrocortisone Solution (Catalog Number H6909)
1.08 mL of Insulin Solution (Catalog Number I9278)
108 µL of Cholera toxin solution (1 mg/mL), prepared by dissolving Catalog Number C8052 in sterile water. Store solution at 2–8 °C.
10.8 µL of EGF solution (1 mg/mL), prepared by dissolving Catalog Number E9644 in 10 mM acetic acid, followed by 0.2 µm filtration. Store the solution in aliquots at –20 °C.

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.

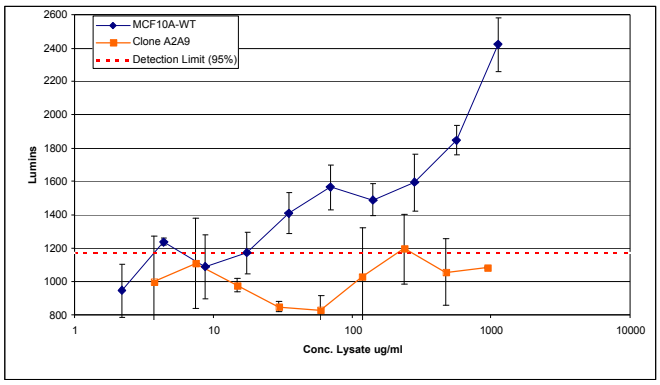
Procedure
Thawing of Frozen Cells
1. Thaw the vial by gentle agitation in a 37 °C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (~2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate 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. Cells prefer to grow in a more dense environment. Allow cells to become 90-95% confluent before attempting to pass.
2. Remove and discard culture medium.
3. Briefly rinse the cell layer with Accutase® (Catalog Number A6964). This cell dissociation solution does not contain mammalian or bacterial-derived products and has been observed to be gentler regarding the dissociation/detachment of this cell line.
4. Add 2.0–3.0 mL of Accutase solution to the flask and incubate at 37 °C for 3–5 minutes (examine the flask every 2 minutes in order to minimize exposure). After the first two minutes, gently agitate cells by hitting the side of the flask with palm of hand. Examine to determine if cells have released.
5. When cells are detached, add 6.0–8.0 ml of Complete Medium and aspirate cells by gentle pipetting.
6. Gently pellet the cells, remove the supernatant, and resuspend to 6–8 mL with prewarmed (37 °C) Complete Medium.
7. Add appropriate aliquots of the cell suspension into new culture vessels. Subcultivation Ratio: 1:2 (or less in order to maintain a higher cell density to promote cell growth).
8. Incubate cultures at 37 °C.

Note: MCF10A parental cells require longer time for digestion/cell release than what is typical. However, it is recommended when passing cells to check every 5 minutes in order to minimize exposure time to Accutase. More information on enzymatic dissociation and subculturing of cell lines is available in the literature.[11]

Results

Figure 2.
Loss of PTEN expression



PTEN expression was examined in wild type MCF10A 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 Number QPBCA). Technical triplicates were examined for each concentration. The ELISA plates were developed using a chemiluminescent peroxidase substrate (Catalog Number CPS260).

References

1. Steck, P.A. et al., Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet., **15** (4), 356–62 (1997).
2. Li, J. et al., PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science, **275** (5308), 1943–7 (1997).
3. Weng, L.P. et al., PTEN Suppresses Breast Cancer Cell Growth by Phosphatase Activity-dependent G_1 Arrest followed by Cell Death. Cancer Res., **59**, 5808–5814 (1999).
4. Saal, L.H. et al., Recurrent gross mutations of the PTEN tumor suppressor gene in breast cancers with deficient DSB repair. Nature Genetics, **40**, 102-107 (2008).
5. Haiman, C.A. et al., Common Genetic Variation at PTEN and Risk of Sporadic Breast and Prostate Cancer. Cancer Epidemiol. Biomarkers Prev., **15**, 1021-1025 (2006).
6. Wallace J.A. et al., Pten in the breast tumor microenvironment: modeling tumor-stroma coevolution. Cancer Res., **15** 71(4), 1203-7 (2011).
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. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC.
9. Hay, R.J. et al., eds. (1992), ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC.
10. Caputo, J.L., Biosafety procedures in cell culture. J. Tissue Culture Methods, **11**, 223-227 (1988).
11. 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 Co. LLC.
ATCC is a registered trademark of American Type Culture Collection.
CRL-10317 is a trademark of American Type Culture Collection.
Accutase is a registered trademark of Innovative Cell Technologies, Inc.

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.

IC,GW,ADM,MAM 10/12-1