

Product Information

CompoZr® Disease Model Cell Lines MCF10A Cells PVRL –/–

Catalog Number **CLLS1211**

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. In order to develop the PVRL knockout cell line, a donor oligonucleotide containing stop codons in all three-frame shifts (see Figure 1) and ~50 bp long homology arms was included when the cells were treated with the ZFNs targeting the beginning of PVRL coding region (N-terminus). As a result, the triple stop insertion was introduced in the gene through homologous recombination in the presence of the donor oligo or deletions were created near the cutting site by non-homologous end joining. Homozygote PVRL4 knockout single cell clones containing either stop codon or deletions in the gene were selected by screening. These 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 site-specific insertion of the triple-stop fragment was introduced in each of the two PVRL4 alleles.

PVRL4 (Nectin-4) is a member of the cell adhesion molecule family that is homologous to PVR/CD155 (the PolioVirus Receptor), named Nectins.¹ All Nectins except Nectin-4 are expressed in epithelial, endothelial, hematopoietic, and neuronal cells in adult tissues.¹ Nectin-4 is mainly expressed during embryogenesis and it is not detected either in normal adult tissues or in serum. Nectin-4 is a tumor-associated antigen and a reliable marker for breast carcinoma, as it is highly expressed in both tumor cell lines and tumors from breast origin.²

Also, the levels of Nectin-4 are elevated in ovarian cancer cell lines and tissue, and in more than half of a series of other types of cancers such as bladder and cervical carcinomas suggesting its diagnostic and therapeutic application to a wide range of tumors.³

Components

MCF10A mutant cell line with PVRL4 knockout
 Catalog Number CLL1211 1 vial

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

1 vial of MCF10A cells contains ~2 × 10⁶ cells.

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

Figure 1.

Site-specific insertion of the triple-stop fragment in PVRL4 Locus in the MCF10A cell line

Wild Type

ATTATGACTGTGCTCCCCAGCTCTGGCAGCACCC
 CTAATGGTGGCTGTCCCTCTCTGCCCCCTGCAGGC
 CGGTGCCCCGCGGGTGAGCTGGAGACCTCAGACG
 TGGTAACTGTGGTGCTGGGCCAGGACGCAAA**CT**
GCCCTGCTTCTACCGAggggac**TCCGGCGAGCAAGT**
GGGGCAAGTGGCATGGGCTCGGGTGGACGCGGG
 CGAAGGCGCCCAGGAAGTAGCGCTACTGCACTCC
 AAATACGGGCTTCATGTGAGCCCGGCTTACGAGGG
 CCGCGTGGAGCAGCCGCCGCCGCCACGCAACCCC
 CTGGACGGCTCAGTGCTCCTGCGCAACGCAGTGC
 AGGCG**GATGAGGGCGAGTACGAGT**G

Alleles 1 & 2 – 31 bp insertion

ATTATGACTGTGCTCCCCAGCTCTGGCAGCACCC
 CTAATGGTGGCTGTCCCTCTCTGCCCCCTGCAGGC
 CGGTGCCCCGCGGGTGAGCTGGAGACCTCAGACG
 TGGTAACTGTGGTGCTGGGCCAGGACGCAAA**CT**
GCCCTGCTTCTACCGAggg**AAGCTTGGGTGAGTAG**
GTAG**TAGTAGGTAG**gac**TCCGGCGAGCAAGTG**
 GGCAAGTGGCATGGGCTCGGGTGGACGCGGGCG
 AAGGCGCCCAGGAAGTAGCGCTACTGCACTCCAAA
 TACGGGCTTCATGTGAGCCCGGCTTACGAGGGCC
 GCGTGGAGCAGCCGCCGCCACGCAACCCCT
 GGACGGCTCAGTGCTCCTGCGCAACGCAGTGACG
 GCG**GATGAGGGCGAGTACGAGTG**

Schematic of the genomic sequence at the target region recognized by the ZFN pair, the resulting nucleotide change, 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 - **UPPER CASE, BOLDED BLUE**

Genotype: homozygous insertion of **AAGCTTGGGTGAGTAGGTAGT** at target's N-terminus creating stop codons that knock out gene expression of PVRL4.

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.

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.⁴⁻⁷

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.

Preparation Instructions

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% CO₂ in air atmosphere.

Medium Components:

DMEM/F12, Catalog Number 51448C

Cholera Toxin from *Vibrio cholerae*,
Catalog Number C8052

Epidermal Growth Factor, Catalog Number E9644

Horse Serum, Catalog Number H1270

Hydrocortisone Solution, 50 µM,
Catalog Number H6909

Insulin Solution, Catalog Number I9278

To make the complete growth medium combine the following:

1. 1 liter of DMEM/F12
2. 108 µL of Cholera toxin solution (1 mg/mL), prepared by dissolving Catalog Number C8052 in sterile water. Store solution at 2–8 °C.
3. 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.
4. 50 mL of horse serum
5. 29 mL of Hydrocortisone Solution, 50 µM
6. 1.08 mL of Insulin Solution

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 culture flask. It is recommended to begin the culture in a 25 cm² flask as the initial adaptation and expansion of the cells is more rapid in a denser environment. 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. 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.⁸

References

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4. 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
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Additional product and technical information can be obtained from the catalog references and the Sigma Life Science Website www.sigma.com/biocells

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