

Product Information

CompoZr® Disease Model Cell Lines MCF10A Cells SMO W535L

Catalog Number **CLLS1110**

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 prepare the SMO W535L SNP, a donor oligonucleotide with a single nucleotide change was included when cells were treated with the ZFN nuclease targeting the SMO site. As a result of homologous recombination in the presence of the donor oligo, this SNP was introduced (see Figure 1). Single cell 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 desired single nucleotide change was introduced in each of the two alleles.

The SMO (Smoothed) gene codes for a G-coupled receptor protein belonging to the Hedgehog pathway.¹ Smoothed is a co-receptor with Patched. In the absence of the Hedgehog ligand, Patched and Smoothed (the signaling partner) are in a complex in the membrane, and the genes downstream (such as the transcription factor Gli) are not active. Thus, Patched either directly or indirectly inhibits SMO activity. Smoothed becomes active as stimulation of the Patched receptor by the Sonic hedgehog ligand leads to subsequent translocation to the primary cilium and results in activation of the Gli protein transactivator. Some mutant forms of SMO cannot translocate from the cell surface and cannot contribute to pathway activation; however, some forms of SMO can function as an oncogene. Activating mutations in SMO have been associated with oncogenesis.²⁻⁴

Components

MCF10A mutant cell line with SMO W535L 1 vial
 SNP insertion
 Catalog No. CLL1110

Parental mammary epithelial cell line 1 vial
 (ATCC® Catalog No. CRL-10317™)
 Catalog No. CLL1040

1 vial of MCF10A cells contains $\sim 2 \times 10^6$ cells.

The cryoprotectant medium used is Cell Freezing Medium-DMSO 1×, Catalog No. C6164.

Figure 1.

Creation of SMO W535L SNP in MCF10A Cells

Site-specific insertion of the W535L mutation at the SMO Locus in the MCF10A cell line

Wild Type

AAAAAGAGAGAGGAAAAGAAAGGAAAGCCTCACCT
GTCTACGTTCCCTCACTGTAGATGTCAGGCCAATG
 TGACCATCGGGCTGCCCACCAAGCAGCCCATCCCT
 GACTGTGAGATCAAGAATCGCCCCGAGCCTTCTGGT
 GGAG**AAGATCAACCTGTTT**gccatgt**TTGGA**ACTGGC
ATCGCCATGAGCACCT**TGG**GTCTGGACCAAGGCCA
 CGCTGCTCATCTGGAGGCGTACCTGGTGCAGGTG
 GGCATGGCAGCCAGCCCCCTCCTGCCCTGCCCGCC
 TCACCCTCAGCCTTGGGACCCCATCTTTAGGTTTT
 GTCGGGTCTCTG**CCTCTAGCACAGCCTTGGT**CAGT
 GGTTCACCGCTGCCCCCT

Alleles 1&2 – SNP W535L integrated

AAAAAGAGAGAGAGGAAAAGAAAGGAAA**GCCTCACCT**
GTCTACGTTCCCTCACTGTAGATGTCAGGCCAATG
 TGACCATCGGGCTGCCACCAAGCAGCCCATCCCT
 GACTGTGAGATCAAGAATCGCCCGAGCCTTCTGGT
 GGAG**AAGATCAACCTGTTT**gcatgt**TTGGA**ACTGGC
ATCGCCATGAGCACC**TTG**GTCTGGACCAAGGCCA
 CGCTGCTCATCTGGAGGCGTACCTGGTGCAGGTG
 GGCATGGCAGCCAGCCCTCCTGCCCTGCCCGCC
 TCACCCTCAGCCTTGGGACCCCATCTTTAGGTTTT
 GTCGGGTCTCTG**CCTCTAGCACAGCCTTGGTC**AGT
 GGTTCACCGCTGCCCCCT

Schematic of the genomic sequence at the target region (exon 9) 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**
 Nucleotide change - **UPPER CASE, BOLDED BLUE**
 (change from G, wild type, to T)
 Codon change - **green highlighted**
 (change from TGG, wild type, to TTG)

Genotype: single nucleotide conversion of G to T (W535L) in exon 9 (converting tyrtophan-535 to leucine in the protein)

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 No. 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.⁵⁻⁸

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 No. 51448C
Cholera Toxin from *Vibrio cholerae*, Catalog No. C8052
Epidermal Growth Factor, Catalog No. E9644
Horse Serum, Catalog No. H1270
Hydrocortisone Solution, 50 µM. Catalog No. H6909
Insulin Solution, Catalog No. 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 No. 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 side of 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 6.0–8.0 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.⁹

References

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

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