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Product Information

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

Catalog Number **CLLS1069**
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 APC 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 are eliminated, in contrast to cell lines with normal expression.

APC is a tumor suppressor gene involved in signalling from the Wnt receptor that leads to activation of the β-catenin transcription coactivator.[1,2] APC and β-catenin are found to be commonly mutated in both sporadic and familial colon cancer.[3,4] When the pathway is not activated, a complex forms in the cytoplasm containing APC, β-catenin, GSK3, Axin, and CK-1.[5] This complex leads to phosphorylation of β-catenin, which drives the protein to degradation through the proteosome. Activation of Wnt signaling leads to inhibition of GSK3 activity allowing stabilization of β-catenin, which then enters the nucleus. Likewise, mutations in APC or β-catenin can lead to activation of the pathway. Once in the nucleus β-catenin binds to the TCF/LEF transcription factor leading to activation of pathways involved in growth regulation of the cell.

While APC mutations that effect β-catenin expression have not been commonly found in breast cancer, increased β-catenin levels have been found in breast cancer and correlate with poorer prognosis.[6,7] Changes in methylation of APC in the seurm of breast cancer patients have been found to be an independent prognostic parameter.[8] These alterations in the Wnt signalling pathway in breast cancer merit further investigation.

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

Figure 1.
Creation of APC Knockout in MCF10A Cells

Site-specific deletion at the APC Locus in MCF10A cell line, Alleles 1 and 2 – 17 bp deletion:

ATAGGTCAGACAATTTTAATACTGGCAACATGACTG
TCCTTTCACCATATTTGAATACTACAGTG**TTACCCA**
GCTCCTCTTCATCAAGAGGAAGCTTAGATAGTTCT
CGTTCTGAAAAAGATAGAAGTTTGGAGAGAGAACG
CGGAATTGGTCTAGGCAACTACCATCCAGCAACAG
AAAATCCAGGAACTTCTTCAAAGCGAGGTTTGCAG
ATCTCCACCACTGCAGCCCAGATTgccaaaGTCATG
GAAGAAGTGTCAGCCATTCATACCTCTCAGGAAGA
CAGAAGTTCTGGGTCTACCACTGAATTACATTGTGT
GACAGATGAGAGAAATGCACTTAGAAGAAGC**TCTG**
CTGCCCATACACATTCAAACACTTACAATTTCACTA
AGTCGGAAAA

Schematic of the genomic sequence at the target region (exon 16) 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 17/del 17 (homozygous)

Components

MCF10A mutant cell line with APC gene knocked out — 1 vial
Catalog No. CLL1069

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

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

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.

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

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, 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.[9-12]

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. 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 the flask and incubate at 37 °C for 15 minutes. This should remove the cells from the cultureware and yield single cells.
4. When cells are detached, 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: MCF10A cells require longer time for trypsin digestion than what is typical. More information on enzymatic dissociation and subculturing of cell lines is available in the literature.[13]

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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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