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Product Information

CompoZr® Disease Model Cell Lines
MCF10A Cells BRAF V600E

Catalog Number **CLLS1107**
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 BRAF-V600E SNP, a donor oligonucleotide with a single nucleotide change was included when cells were treated with the ZFN nuclease targeting the BRAF 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 BRAF gene codes for a serine/threonine-protein kinase. It is known as a proto-oncogene and shown to be mutated in a number of human cancers.[1] One of the most common mutations of BRAF is the single nucleotide modification converting the thymidine at position 1799 in the genomic sequence to alanine (T1799A).[2] This base change results in the amino acid change at position 600 of the protein from valine to glutamine (V600E). The V600E mutation has been strongly associated with melanoma and colon cancer and has been shown to be present in a number of other cancers.[3,4] Studies suggest that the V600E mutation alone is not sufficient to initiate carcinogenesis, but in concert with other oncogenic changes, has been described as a driver mutation.[5,6] It has been suggested that investigation of the BRAF in addition to KRAS and PIK3CA status in breast cancer may prove beneficial in predicting the response to therapeutic agents such as everolimus.[7,8]

For further information, go to CompoZr Breast Cancer Cell Lines at www.sigma.com/biocells

Components

MCF10A mutant cell line with BRAF V600E SNP insertion Catalog No. CLL1107	1 vial
Parental mammary epithelial cell line (ATCC® Catalog No. CRL-10317™) Catalog No. CLL1040	1 vial

1 vial of MCF10A cells contains ~2×10^6 cells.

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

Figure 1.
Creation of BRAF V6000E SNP in MCF10A Cells

Site-specific insertion of the V600E-SNP at the BRAF Locus in the MCF10A cell line

Wild Type

ATTAGATCTCTTACCTAAACTCTTCATAA**TGCTTGCT**
CTGATAGGAAAATGAGATCTACTGTTTTCCTTTACT
TACTACACCTCAGATATATTTCTTCATGAAGACCTC
ACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGTG
AAATCTCGATGGAGTGGGTCCCATCAGTTTG**AACA**
GTTGTCTGGATCCATtttgt**GGATGGTAAGAA**TTGAG
GCTATTTTTCCACTGATTAAATTTTTGGCCCTGAGA
TGCTGCTGAGTTACTAGAAAGTCATTGAAGGTCTCA
ACTATAGTATTTTCATAGT**TCCCAGTATTCACAAAA**
ATCAGTGTTCTTATTTTTTATGTAAATAGATTTTTTA
ACTTTTTTCTTTACCCTTAAAACGAATATTTTGAAAC
CAG

Alleles 1&2 – V6000E

ATTAGATCTCTTACCTAAACTCTTCATAA**TGCTTGCT**
CTGATAGGAAAATGAGATCTACTGTTTTCCTTTACT
TACTACACCTCAGATATATTTCTTCATGAAGACCTC
ACAGTAAAAATAGGTGATTTTGGTCTAGCTACAGAG
AAATCTCGATGGAGTGGGTCCCATCAGTTTG**AACA**
GTTGTCTGGATCCATtttgt**GGATGGTAAGAA**TTGAG
GCTATTTTTCCACTGATTAAATTTTTGGCCCTGAGA
TGCTGCTGAGTTACTAGAAAGTCATTGAAGGTCTCA
ACTATAGTATTTTCATAGT**TCCCAGTATTCACAAAA**
ATCAGTGTTCTTATTTTTTATGTAAATAGATTTTTTA
ACTTTTTTCTTTACCCTTAAAACGAATATTTTGAAAC
CAG

Schematic of the genomic sequence at the target region (exon 15) 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 T, wild type, to A)
Codon change - green highlighted
(change from GTG, wild type, to GAG)

Genotype: single nucleotide conversion of T to A (T1799A) in exon 15 (converting valine-600 to glutamine 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.[9-12]

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% CO_2 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 cm^2 culture flask. It is recommended to begin the culture in a 25 cm^2 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% 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 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.
3. 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.
4. When cells are detached, add 6.0–8.0 ml of Complete Medium and aspirate cells by gentle pipetting.
5. Gently pellet the cells, remove the supernatant, and resuspend to 6- 8 mL with prewarmed (37 °C) Complete Medium.
6. 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).
7. 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.[13]

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