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Product Information

CompoZr® Disease Model Cell Lines
MCF10A Cells ESR2 –/–

Catalog Number **CLLS1075**
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. By providing a donor oligo containing homologous arms flanking stop codons, homologous recombination resulted in the introduction of stop codons in the first coding exon (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 target gene is disrupted and the corresponding functional protein expression eliminated, in contrast to cell lines expressing fully functional ESR2.

Estrogen receptor 2 (ESR2) was first cloned from rat cells in 1996.[1,2] This gene encodes a protein belonging to the estrogen receptor family and is a component of the superfamily of nuclear receptor transcription factors. The ESR2 gene consists of eight coding exons, resulting in two isoforms. Full length product consists of 530 amino acids, with a second shorter isoform of 495 amino acids. The two isoforms are identical with the exception of the C-terminus portion. In the second, shorter isoform exon 8 of the longer isoform is replaced with 26 unique amino acids. ER-mediated gene expression results from the direct binding of dimerized ER regions of the DNA known as estrogen response elements (ERE). While a role for ESR2 in the development of breast cancer has been indicated, the specific role that polymorphic sites in this gene play in breast cancer ontology have yet to be fully determined.[3-5]

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

Components

MCF10A mutant cell line with ESR2 gene knocked out, Catalog No. CLL1075	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 ESR2 Knockout in MCF10A Cells

Site-specific gene silencing at the ESR2 Locus in MCF10A cell line

Wild-type

G**ACCTTTGTGCCTCTTCTTGC**AAGGTGTTTTCTCAG
CTGTTATCTCAAGAC**ATG**GATATAAAAAACTCACCA
TCTAGCCTTAATTCTCCTTCCTCCTACAACTGCAGT
CAATCCATCTTACCCCTGGAGCACGGCTCCATATA
CATACCTTCCTCCTATGTAGACAGCCACCATGAATA
TCCAGCCATGACATTCTATAGCCCTGCTGTGATGA
ATTACAGCATT**CCCAGCAATGTCACT**aacttg**GAAGG**
TGGGCCTGGTCGGCAGACCACAAGCCCAAATGTG
TTGTGGCCAACACCTGGGCACCTTTCTCCTTTAGT
GGTCCATCGCCAGTTATCACATCTGTATGCGGAAC
CTCAAAAGAGTCCCTGGTGTGAA**GCAAGATCGCTA**
GAACACACCTTACCT

Allele 1 – stop oligo incorporated:

ACCTTTGTGCCTCTTCTTGCAAGGTGTTTTCTCAGC
TGTTATCTCAAGAC**ATG**GATATAAAAAACTCACCAT
CTAGCCTTAATTCTCCTTCCTCCTACAACTGCAGTC
AATCCATCTTACCCCTGGAGCACGGCTCCATATAC
ATACCTTCCTCCTATGTAGACAGCCACCATGAATAT
CCAGCCATGACATTCTATAGCCCTGCTGTGATGAA
TTACAGCATTCCCAGCAATGTCACTAACTAGTAATG
AGGGCCTGGTCGGCAGACCACAAGCCCAAATGTG
TTGTGGCCAACACCTGGGCACCTTTCTCCTTTAGT
GGTCCATCGCCAGTTATCACATCTGTATGCGGAAC
CTCAAAAGAGTCCCTGGTGTGAA**GCAAGATCGCTA
GAACACACC**

Allele 2 – stop oligo incorporated & 1 bp replacement:

ACCTTTGTGCCTCTTCTTGCAAGGTGTTTTCTCAGC
TGTTATCTCAAGAC**ATG**GATATAAAAAACTCACCAT
CTAGCCTTAATTCTCCTTCCTCCTACAACTGCAGTC
AATCCATCTTACCCCTGGAGCACGGCTCCATATAC
ATACCTTCCTCCTATGTAGACAGCCACCATGAATAT
CCAGCCATGACATTCTATAGCCCTGCTGTGATGAA
TTACAGCATTCCCAGCAATGTCACTAGCTAGTAATG
AGGGCCTGGTCGGCAGACCACAAGCCCAAATGTG
TTGTGGCCAACACCTGGGCACCTTTCTCCTTTAGT
GGTCCATCGCCAGTTATCACATCTGTATGCGGAAC
CTCAAAAGAGTCCCTGGTGTGAA**GCAAGATCGCTA
GAACACACC**

Schematic of the genomic sequence at the target region (exon 3) recognized by the ZFN pair; the resulting donor oligo insertion, and the CEL-I primer sequences:

CEL-I Primers - **Bolded & underlined**
ZFN binding site - **UPPER CASE, BOLDED RED**
zfn cut site - lower case red
Exon 3, start codon - **yellow highlighted, BOLDED RED**
Donor oligo with stop codon - blue highlighted
Single nucleotide replacement - purple highlight

Genotype: stop codon insertion in exon 3 (the first coding exon).

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.[6-9]

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 liter of DMEM/F12
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.
50 mL of horse serum
29 mL of Hydrocortisone Solution, 50 μM
1.08 mL of Insulin Solution

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.

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 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. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924).
3. Then, add 2.0–3.0 mL of Trypsin-EDTA solution to the flask and incubate at 37 °C for 15 minutes (examine the flask every 5 minutes in order to minimize exposure to the trypsin-EDTA). 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. Gently pellet the cells, remove the supernatant, and resuspend to 6–8 mL with Complete Medium.
6. Add appropriate aliquots of the cell suspension into new culture vessels.
   Subcultivation Ratio: 1:2 to 1:4
7. 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.[10]

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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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