SIGMA-ALDRICH®

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Product Information

CompoZr® Disease Model Cell Lines
MCF10A Cells H2AFX –/–

Catalog Number **CLLS1061**
Storage Temperature –196 °C (liquid nitrogen)

TECHNICAL BULLETIN

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 H2AFX 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 resulting protein are disrupted, in contrast to cell lines with normal expression.

Histone 2A family member X (H2AFX) is one of the several genes coding for histone H2A[1]. The compact chromosomal structure of DNA in eukaryotes results from the interaction of DNA with various histones, which are basic nuclear proteins. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form an octamer core, around which approximately 146 bp of DNA is wrapped. This core structure is called the nucleosome. The product of H2AFX (a.k.a. H2A.X; H2A/X; H2AX; H2AX) contributes to histone-formation and therefore to the structure of DNA. The gene coding H2AFX is located at 11q23.3[1]. One of the first cellular responses to the introduction of double-strand breaks is the phosphorylation of H2AFX, one of three types of histone H2A molecules in eukaryotic cells[2]. Serine 139 in the unique carboxy-terminal tail of H2AFX is phosphorylated within 1 to 3 minutes after damage, and the number of H2AFX molecules phosphorylated increases linearly with the severity of the damage. While the introduction of double-strand breaks elicits the H2AFX response, other types of DNA damage, such as UV irradiation, do not[1]. H2AFX is involved in DNA damage checkpoint; double-strand break repair via homologous recombination; DNA repair; double-strand break repair; DNA recombination; nucleosome assembly; response to DNA damage stimulus; cell cycle; meiosis; spermatogenesis; cell aging[3-6].

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

Components

MCF10A mutant cell line with H2AFX gene knocked out, Catalog No. CLL1061	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 H2AFX Knockout in MCF10A Cells

Site-specific deletion at the H2AFX Locus in MCF10A cell line.

Alleles 1&2 – 5 bp deletion:
GCGACACCCAAGGGTTAACCGCAACCAACCGG**AGGCGGGTATTGGAGAAAAG**AGCCAATCAGGAGGGCGCAGAGGTGTGTCCTGGGGGCTTATAAAGGCGGCCTCGCGGCGCGCGCGCGACAGCAGTTACACTGCGGCGGGCGTCTGTTCTAGTGTTTGAGCCGTCGTGCTTCACCG**GTCTACCTCGCTAGCATG**tcgggcc**GCGGCAAGACTGGCGGCA**AGGCCCGCGCCAAGGCCAAGTCGCGCTCGTCGCGCGCCGGCCTCCAGTTCCCAGTGGGCCGTGTACACCGGCTGCTGCGGAAGGGCCACTACGCCGAGCGCGTTGGCGCCGGCGCGCCAGTGTACCTGGCGGCAGTGCTGGAGTACCTCACCGCTGAGATCCTGGAGCTGGCGGGCAATGCGGCCCGCGA**CAACAAGAAGACGCGAATCA**TCCCCCGCCACCTGCAGCTGGCCATCCGCAACGACGAGGA
Schematic of the genomic sequence at the target region (exon 1) 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 5/del 5 (homozygous)

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[7-10].

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.

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 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.

Sub-culturing 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[11].

References

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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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