

Product Information

CompoZr® Disease Model Cell Lines SW48 Cells HIF1a -/-

Catalog Number **CLLS1098**

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 HIF1a locus (see Figures 1a and 1b). Single cell knockout clones were isolated and followed for more than twenty passages to establish stable cell lines.

The targeted gene in this cell line is triploid due to a duplication and translocation of a portion of chromosome 14 to chromosome 22. Demonstrating the utility of ZFN-mediated gene knockout technology, it 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.

Hypoxia-Inducible Factor 1-alpha (HIF1a) plays an essential role in cells responding to low oxygen or hypoxic conditions.¹ HIF1a expression appears essential to tumor angiogenesis² and overexpression has been associated with poor prognosis in colorectal cancer.³ The availability of cells lines with stably repressed or knocked out HIF1a expression allow the investigation of the potential roles of therapeutic agents.⁴

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

Components

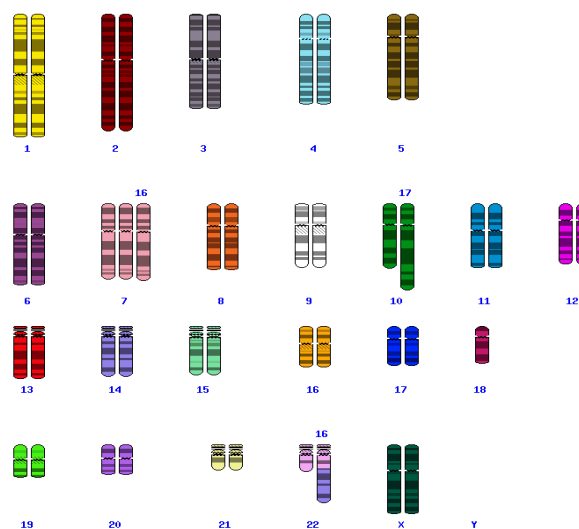
SW48 mutant cell line with HIF1a gene knocked out 1 vial
 Catalog No. CLL1098

Parental colon adenocarcinoma cell line 1 vial
 (ATCC® Catalog No. CCL-231™)
 Catalog No. CLL1008

1 vial of modified SW48 cells contains ~2 × 10⁶ cells.

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

Figure 1a.
 Creation of HIF1a Knockout in SW48 Cells



HIF1a is triploid in SW48 cell line: location – 14q21-q24, der(22)t(14;22)(q12;qter) (from NCBI SKY/M-FISH database).

Figure 1b. Site-specific deletion at the HIF1a Locus in SW48 cell line (Clone A4)

Allele 1 – 4 bp insertion:

AATGCTCCATTACCCACCGCTGAAACGCCAAAGC
CACTTCGAAGTAGTGCTGACCCTGCACTCAATCAA
GAAGTTGCATTAATAAATTAGAACCAAATCCAGAGTCA
CTGGAACCTTTCTTTTACCATGCCCCAGATTCCAGGAT
CAGAC**ACCTAGTCCTTcCGATcgatgGAAGCACTAG**
ACAAAGTTACCTGAGGTAGGTGTCATGATATAAT
CAGAAAGGGACAACCTTTTACGATTTTAAACATTCAAGA
ATGTATTTATAAGTTTGATTCAAACACTCATTTGAAC
CACAAATTACATTTGTGTGTGTGTTTGAATTTTAGC
ACTTTAAATTATTGCAAGAGCTACT**TGCCTAACCTA**
GACCTGAGCA

Allele 2 – 29 bp deletion:

AATGCTCCATTACCCACCGCTGAAACGCCAAAGC
CACTTCGAAGTAGTGCTGACCCTGCACTCAATCAA
GAAGTTGCATTAATAAATTAGAACCAAATCCAGAGTCA
CTGGAACCTTTCTTTTACCATGCCCCAGATTCCAG**GAT**
CAGACACCTAGTCCTTccgatgGAAGCACTAGACAA
AGTTACCTGAGGTAGGTGTCATGATATAATCAGA
AAGGGACAACCTTTTACGATTTTAAACATTCAAGAATGT
ATTTATAAGTTTGATTCAAACACTCATTTGAACCACA
AATTACATTTGTGTGTGTGTTTGAATTTTAGCACTTT
AAAATTATTGCAAGAGCTACT**TGCCTAACCTAGACC**
TGAGCA

Allele 3 – 1 + 6 bp deletion:

AATGCTCCATTACCCACCGCTGAAACGCCAAAGC
CACTTCGAAGTAGTGCTGACCCTGCACTCAATCAA
GAAGTTGCATTAATAAATTAGAACCAAATCCAGAGTCA
CTGGAACCTTTCTTTTACCATGCCCCAGATTCCAGGAT
CAGAC**ACCTAGTCCTTccgatgGAAGCACTAGACAA**
AGTTACCTGAGGTAGGTGTCATGATATAATCAGA
AAGGGACAACCTTTTACGATTTTAAACATTCAAGAATGT
ATTTATAAGTTTGATTCAAACACTCATTTGAACCACA
AATTACATTTGTGTGTGTGTTTGAATTTTAGCACTTT
AAAATTATTGCAAGAGCTACT**TGCCTAACCTAGACC**
TGAGCA

Schematic of the genomic sequence at the target region 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 RED**

ZFN cut site – **lower case red**

Deletion – **yellow highlighted**

Insertion – **green highlighted**

Cell Line Description

Organism: *Homo sapiens* (human)

Tissue: adenocarcinoma; colorectal

Age: 82 years

Gender: Female

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile

Short Tandem Repeat (STR) analysis:

Amelogenin: X

CSF1PO: 9, 10

D13S317: 11, 12

D16S539: 11,13

D5S818: 10, 14

D7S820: 9, 10

TH01: 6,9.3

TPOX: 8

vWA: 18, 20, 21

Parental Cell Line: ATCC Catalog No. CCL-231

Note: Please see CCL-231 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, SW48, 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.⁵⁻⁷

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) – high glucose supplemented with 10% v/v fetal bovine serum (FBS), 2 mM L-glutamine and 1 mM sodium pyruvate. This medium is formulated for use with a 5% CO_2 in air atmosphere.

Medium Components:

DMEM, Catalog No. D5671

FBS, Catalog No. F4135

L-glutamine, Catalog No. G7513

Sodium pyruvate, Catalog No. S8636

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 $\sim 125 \times g$ 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.

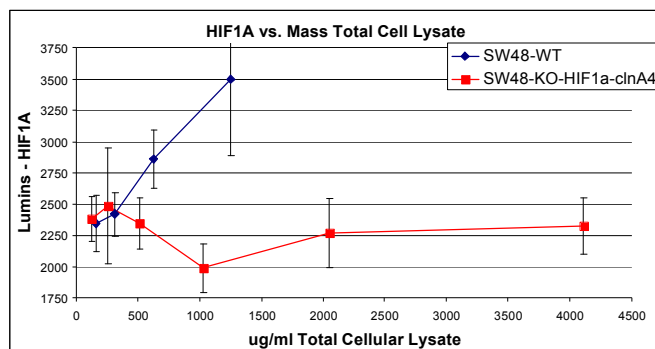
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Trypsin-EDTA solution (Catalog No. T3924)
3. Add 2.0–3.0 mL of Trypsin-EDTA solution to flask and incubate at 37°C for 10 minutes to detach the cells.
4. When cells are detached, add 6.0–8.0 mL of Complete Medium and aspirate 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: More information on enzymatic dissociation and subculturing of cell lines is available in the literature.⁸

Results

Figure 2.

Loss of HIF1a expression



HIF1a expression was examined in wild type SW48 and the knockout cloned line using an enzyme-linked immunosorbent assay (a modification of R&D Systems DYC1935-2). Briefly, both cell populations were treated 16 hours prior to harvest with medium containing reduced serum (0.1%v/v) and 0.15 mM cobalt chloride in order to mimic hypoxic conditions and induce HIF1a expression. The nuclear fraction was collected for each population and the total protein concentration determined by BCA assay (Catalog No. QPBCA). In order to determine levels of HIF1a, a standard curve was generated using recombinant HIF1a (R&D Systems, Catalog No. 841691), and a detection limit of 0.16 ng/mL was determined. Technical triplicates were examined for each concentration. The ELISA plates were developed using a chemiluminescent peroxidase substrate (Catalog No. CPS260).

References

1. Ratcliffe, P.J., From erythropoietin to oxygen: hypoxia-inducible factor hydroxylases and the hypoxia signal pathway. *Blood Purif.*, **20**(5), 445-450 (2003).
2. Yang, Q.C., Inhibition of hypoxia-induced angiogenesis by trichostatin A via suppression of HIF-1a activity in human osteosarcoma. *J. Exp. Clin. Cancer Res.*, **25**(4), 593-599 (2006).
3. Baba, Y. et al., HIF1A overexpression is associated with poor prognosis in a cohort of 731 colorectal cancers. *Am. J. Pathol.*, **176**(5), 2292-2301 (2010).
4. Fels, D.R. et al., Preferential cytotoxicity of bortezomib toward hypoxic tumor cells via overactivation of endoplasmic reticulum stress pathways. *Cancer Res.*, **68**(22), 9323-9330 (2008).
5. Hay, R.J. et al., eds., *ATCC Quality Control Methods for Cell Lines*. 2nd edition, Published by ATCC (1992).
6. Caputo, J.L., Biosafety procedures in cell culture. *J. Tissue Culture Methods*, **11**, 223-227 (1988).
7. Centers for Disease Control (1999), *Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 93-8395*. U.S. Dept. of Health and Human Services; 4th Edition U.S. Government Printing Office Washington D.C. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm
8. Freshney, R.I., Chapter 10 in *Culture of Animal Cells*, a manual of Basic Technique by, 3rd edition, published by Alan R. Liss, (NY, NY: 1994).

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

Please see the Label License Agreement (LLA) for further details regarding the use of this product. The LLA is available on our Web site at www.wherebiobegins.com/biocells

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