3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

CompoZr® Disease Model Cell Lines
MCF10A Cells MAP3K8 P461L

Catalog Number **CLLS1118**
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 MAP3K8 P461L SNP, a donor oligonucleotide with a single nucleotide change was included when cells were treated with the ZFN nuclease targeting the MAP3K8 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 MAP3K8 (mitogen activated protein kinase kinase kinase) gene codes for a serine/threonine-protein kinase. The gene was initially identified for its transforming properties as a C-terminally truncated oncogene. It plays a crucial role in the activation of several signal transduction pathways, which themselves activate specific map kinases.[1-6] The activation of these subsequent map kinases have been identified in many cases as requisite in tumorigenesis.[7-11] The half-life and kinase activityof MAP3K8 is increased in instances of C-terminal truncations, thereby potentiating its mitogenic effects.[4,12,] Additionally, overexpression of MAP3K8 has been noted in some breast cancers, with the C-terminal point mutation, P461L, identified in some of these instances.[13,14]

Components

MCF10A mutant cell line with MAP3K8 P461L SNP insertion Catalog No. CLL1118	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 MAP3K8 P461L SNP in MCF10A Cells

Site-specific insertion of the P461L-SNP at the MAP3K8 Locus in the MCF10A cell line

Wild Type

TATGTCATATTATGTGAACTTAGCCATAGTGTT**CAA**
AGACCCATCTTTCACGCTTAAGACACTGTTTTATTA
GGCCCCAAAGATTTATTTCACTGCAGAAGGAACAG
GTATTTATCATTTGACACGTTTTCTTGTTACTTACTT
TGTAATGTTTTCCTTTTCAGATTCTTCGTGCACAGG
AAGCACCGAGGAATCTGAGATGCTCAAGAGGCAAC
G**CTCTCTCTACATC**gacctc**GGCGCTCTGGCTGGC**TA
CTTCAATCTTGTTCGGGGACCACCAACGCTTGAAT
ATGGCTGAAGGATGCCATGTTTGCTCTAAATTAAGA
CAGCATTGATCTCCTGGAGGCTGGTTCTGCTGCCT
CTACACAGGGGCCCTGTACAGTGAATGGTGCCATT
TTCGAAGGAGCAGTGTGACCTCCTGTGACCCGTGA
ATGTGCCTCCAAGCGGCCCTGTGTGTTTGACATGT
GAAGCTATTTGAT**ATGCACCAGGTCTCAAGGTT**CT
CATTTCTCAGGTGACGTGATTCTAAGGCAGGA

Alleles 1&2 – SNP P461L integrated

TATGTCATATTATGTGAACTTAGCCATAGTGTT**CAA**
AGACCCATCTTTCACGCTTAAGACACTGTTTTATTA
GGCCCCAAAGATTTATTTCACTGCAGAAGGAACAG
GTATTTATCATTTGACACGTTTTCTTGTTACTTACTT
TGTAATGTTTTCCTTTTCAGATTCTTCGTGCACAGG
AAGCACCGAGGAATCTGAGATGCTCAAGAGGCAAC
G**CTCTCTCTACATC**gacctc**GGCGCTCTGGCTGGC**TA
CTTCAATCTTGTTCGGGGACTACCAACGCTTGAATA
TGGCTGAAGGATGCCATGTTTGCTCTAAATTAAGAC
AGCATTGATCTCCTGGAGGCTGGTTCTGCTGCCTC
TACACAGGGGCCCTGTACAGTGAATGGTGCCATTT
TCGAAGGAGCAGTGTGACCTCCTGTGACCCGTGAA
TGTGCCTCCAAGCGGCCCTGTGTGTTTGACATGTG
AAGCTATTTGAT**ATGCACCAGGTCTCAAGGTT**CTC
ATTTCTCAGGTGACGTGATTCTAAGGCAGGA

Schematic of the genomic sequence at the target region (exon 9) 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 C, wild type, to T)
Codon change - green highlighted
(change from CCA, wild type, to CTA)

Genotype: single nucleotide conversion of C to T (P461L) in exon 9 (converting proline-461 to leucine 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.[15-18]

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 cm2 culture flask. It is recommended to begin the culture in a 25 cm2 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.
4. 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.
5. When cells are detached, add 6.0–8.0 mL of Complete Medium and aspirate cells by gentle pipetting.
6. Gently pellet the cells, remove the supernatant, and resuspend to 6.0–8.0 mL with prewarmed (37 °C) Complete Medium.
7. 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).
8. 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.[19]

References

1. Chiariello, M., et al. Multiple mitogen-activated protein kinase signaling pathways connect the cot oncoprotein to the c-jun promoter and to cellular transformation. *Mol. Cell. Biol.*, **20**(5), 1747-58 (2000).
2. Dumitru, C.D., et al. TNF-alpha induction by LPS is regulated posttranscriptionally via a Tpl2/ERK-dependent pathway. *Cell*, **103**(7), 1071-83 (2000).
3. Hagemann, D., et al. Cot protooncoprotein activates the dual specificity kinases MEK-1 and SEK-1 and induces differentiation of PC12 cells. *Oncogene*, **18**(7), 1391-1400 (1999).
4. Ceci, J. D., et al. Tpl-2 is an oncogenic kinase that is activated by carboxy-terminal truncation. *Genes Dev.*, **11**(6), 688-700 (1997).
5. Patriotis, C., et al. Tpl-2 acts in concert with Ras and Raf-1 to activate mitogen-activated protein kinase. *Proc. Natl. Acad. Sci. USA*, **91**(21) 9755-9 (1994).
6. Salmeron, A., et al. Activation of MEK-1 and SEK-1 by Tpl-2 proto-oncoprotein, a novel MAP kinase kinase kinase. *EMBO J.*, **15**(4), 817–826 (1996).
7. Ballester, A., et al. 1998. Cot kinase activates tumor necrosis factor-alpha gene expression in a cyclosporin A-resistant manner. *J. Biol. Chem.*, **273**(23), 14099–106.
8. Patriotis, C., et al. Tumor progression locus 2 (Tpl-2) encodes a protein kinase involved in the progression of rodent T-cell lymphomas and in T-cell activation. *PNAS*, **90**(6), 2251-5 (1993).
9. Sanchez-Gongora, E., et al. COT kinase proto-oncogene expression in T cells: implication of the JNK/SAPK signal transduction pathway in COT promoter activation. *J. Biol. Chem.*, **275**, 31379-86 (2000).
10. Tsatsanis, C., et al. Tpl-2 induces IL-2 expression in T-cell lines by triggering multiple signaling pathways that activate NFAT and NF-kappaB. *Oncogene*, **17**(20), 2609-2618 (1998).
11. Velasco-Sampayo, A. and Alemany, S., p27kip protein levels and E2F activity are targets of Cot kinase during G1 phase progression in T cells. *J. Immunol.* **166**(10), 6084-90 (2001).
12. Gándara, M.L., et al. 2003. The COOH-terminal domain of wild-type Cot regulates its stability and kinase specific activity. *Mol Cell Biol*. **20**(23) 7377-90 (2003).
13. Sourvinos, G., et al. Overexpression of the Tpl-2/Cot oncogene in human breast cancer. *Oncogene* **18**(35) 4968–73 (1999).
14. Ding, L. et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. *Nature* **464**(7291) 999-1005 (2010).
15. 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
16. Fleming, D.O. et al., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC.
17. Hay, R.J. et al., eds. (1992), ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC.
18. Caputo, J.L., Biosafety procedures in cell culture. J. Tissue Culture Methods, **11**, 223-227 (1988).
19. 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 references and the Sigma Life Science Website www.sigma.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 http://www.sigmaaldrich.com/content/sigma-aldrich/areas-of-interest/life-science/zinc-finger-nuclease-technology/zfn-license-agreement.html

These cells are distributed for research purposes only. Sigma Life Science requires that individuals contemplating commercial use of any cell line first contact us to negotiate an agreement. Third party distribution of this cell line is prohibited.

CompoZr is a registered trademark of Sigma-Aldrich Co. LLC.
ATCC is a registered trademark of American Type Culture Collection.
CRL-10317 is a trademark of American Type Culture Collection.

GW,ADM,PHC 07/12-1