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Product Information

Safe Harbor Landing Pad Cell Lines
A549 Cancer Cells

Catalog Number **CLL1220**
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 naturally occurring proteins that can be engineered to bind DNA at a sequence-specific location and create a double strand break (www.sigma.com/zfn). 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 typically produces small modifications (indels) at the targeted locus that may result in a functional knockout. Single cell clones are then isolated, tested for the desired modification, and expanded to establish stable cell lines.

The A549 cell line is a human lung carcinoma cell line isolated in 1972 from a lung tumor of a male patient suffering from carcinoma. The cells possess a hypotriploid karyotype.

The homologous recombination pathway was used to insert the Landing Pad construct into the AAVS1 Safe Harbor locus resulting in expression of mKATE2. A donor construct containing a fluorescent reporter gene (mKATE2) flanked by sequences homologous to the regions on either side of the genomic target site was nucleofected into the A549 cells along with ZFNs designed to cut near the genomic target site (see Figure1). Integration resulted in endogenous expression of the fluorescent protein mKATE2.

Figure 1.

Illustration of Landing Pad construct inserted in AAVS1 Safe Harbor locus. The EF1α promoter and mKATE2 gene are flanked and separated by lox sequences for easy recombination by the Cre protein.

Knockin cells were sorted into single cells by flow cytometry and then expanded into clonal populations. Testing of these clones was used to select a Landing Pad clone that contained a single copy of the Landing Pad construct as a stable cell line.

Junction PCR, digital droplet PCR, and fluorescent analysis showed that the Landing Pad construct is inserted into a single allele in the selected clone.

mKATE2 can easily be exchanged for a payload of the user's choice using Cre recombinase and a targeting vector with appropriate lox sites (see Figure 2). Cells can then be sorted via fluorescence-activated cell sorting (FACS) for loss of mKATE2 expression as a surrogate for successful integration of the targeting vector. Approximately 7-10 days are required for loss of the mKATE2 signal in successfully targeted cells.

Figure 2.

Exchange of Landing Pad payload using Cre recombinase and targeting vector.

Components
This product is a cryovial containing 1 million A549 cells with the Landing Pad construct knocked in.
Catalog Number CLL1223

The cryoprotectant medium used is CryoStor® cell cryopreservation medium containing 10% DMSO
Catalog Number C2874

Cell Line Description
Organism: *Homo sapiens* (human)
Tissue: Carcinoma; Lung
Age: 58 years
Gender: Male
Ethnicity: Caucasian
Morphology: Epithelial
Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X, Y
CSF1PO: 10, 12
D13S317: 11
D16S539: 11,12
D5S818: 11
D7S820: 8, 11
TH01: 8,9.3
TPOX: 8, 11
vWA: 14

The STR profile of this cell line matches that of its parental cell line ATCC® Catalog Number CCL-185.
Note: Please see CCL-185 product datasheet from ATCC for additional information about the origin of these cell lines.

Precautions and Disclaimer
For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

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 submerged 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.

Procedures
Medium Preparation Instructions
The base medium for this cell line is RPMI modified to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, and 1,500 mg/L sodium bicarbonate.
Complete Medium: To make the complete growth medium, add fetal bovine serum to a final concentration of 10%.

Thawing of Frozen Cells
1. Thaw the vial by gentle agitation in a 37 °C water bath (~2 minutes). To reduce the possibility of contamination, keep the O-ring and cap out of the water.
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 mL of Complete Medium and spin at 125 × *g* for 5–7 minutes.
4. Re-suspend 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 Number T3924)
3. Add 2.0–3.0 mL of Trypsin-EDTA solution to flask and incubate at 37 °C for 6 minutes to detach the cells.
4. 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.
   Sub-cultivation Ratio: 1:4 to 1:20.
6. Incubate cultures at 37 °C.

Gene Exchange
Reagents and Equipment Required but Not Provided:

Cre recombinase mRNA
Targeting vector
FACS machine/analyzer
Cell Culture media components
Nucleofector and nucleofection solution
(Alternatively, transfection may be used with appropriate reagents and protocol to introduce the Cre mRNA and targeting DNA into the Landing Pad cells.)

Target Vector Design
LoxN Sequence:
ATAACTTCGTATAGTATACCTTATACGAAGTTAT
Lox2272 Sequence:
ATAACTTCGTATAGGATACTTTATACGAAGTTAT
LoxP Sequence:
ATAACTTCGTATAGCATACATTATACGAAGTTAT

Exchanging the promoter
In order to exchange the EF1A promoter for a different promoter, the donor construct should be designed such that there is an upstream LoxN site followed by the promoter to be inserted, followed by a Lox2272 site.

Exchanging the cDNA
In order to exchange the cDNA for a different cDNA, the donor construct should be designed such that there is an upstream Lox2272 site, followed by the cDNA to be inserted, followed by a LoxP site.

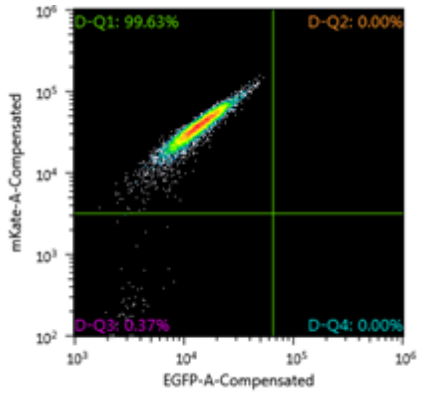
Exchanging the promoter and cDNA
In order to exchange the EF1A promoter and cDNA, the donor construct should be designed such that there is an upstream LoxN site, followed by the sequence to be inserted, followed by a LoxP site.

Gene Exchange Procedure
Complete all steps aseptically

1. Thaw Cre mRNA on ice and maintain stocks on ice throughout procedure.
2. Aliquot 5 μg of targeting vector DNA per reaction into 1.5 mL Eppendorf® tubes, leave one tube empty for a Cre only control.
3. Collect appropriate number of Landing Pad cells from culture, ~1×10^6 per exchange reaction.
4. Spin down cells at 125 × *g* for 5–7 minutes and aspirate medium from cell pellet following centrifugation.
5. Re-suspend cell pellet(s) in nucleofection solution according to manufacturer's protocol.
   Note: Alternatively, transfection may be used with appropriate reagents and protocol to introduce the Cre mRNA and targeting DNA into the Landing Pad cells.
6. Aliquot 5 μg of thawed Cre mRNA into each 1.5 mL Eppendorf tube already containing donor DNA and the empty control tube.
7. Add appropriate volume of cell pellet/nucleofection solution slurry into each 1.5 mL Eppendorf tube and gently mix by pipetting.
8. Transfer each reaction to a nucleofection cuvette.
9. Nucleofect all reactions using appropriate manufacturer's protocol.
10. Plate each reaction in 2 mL of Complete Medium in one well of a sterile, 6 well plate and place at 37 °C.
11. Analyze via FACS for loss of mKATE2 expression 7-10 days post-nucleofection.
12. FACS on the starting Landing Pad population prior to exchange should be used to set appropriate gates (see Figure 3). The Cre only control may show a slight increase in the mKATE2 negative population (see Figure 4).
13. If the desired payload contains a fluorescent marker, enrich for this population (see Figure 5, quadrant 4). If there is no fluorescent tag on the integrant, Landing Pad cells can simply be enriched for loss of mKATE2 signal. Approximately 13% of the mKATE2 negative population had successful Cre-mediated exchange of mKATE2 for the targeting vector in test experiments (see Figure 5 and Equation 2).

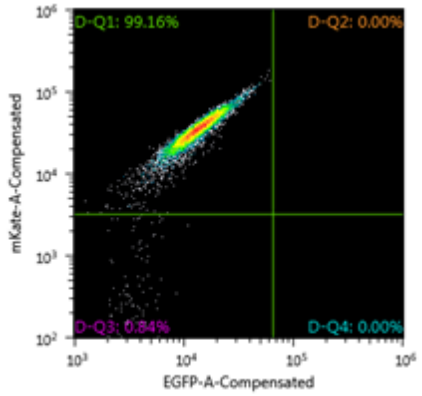
Results

Figure 3.



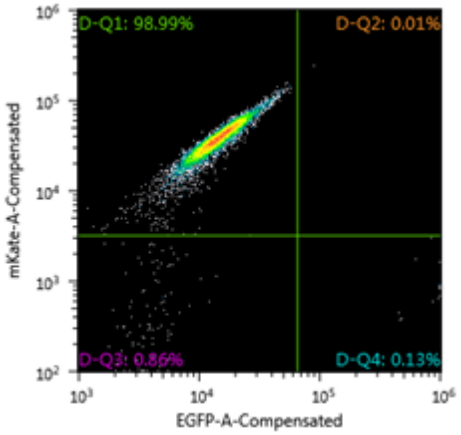
FACS analysis of A549 Landing Pad clone prior to Cre-mediated exchange.

Figure 4.



FACS analysis of A549 Landing Pad clone Cre-only nucleofection control, one week post-nucleofection.

Figure 5.



FACS analysis of A549 Landing Pad clone nucleofected with Cre mRNA and targeting DNA vector containing EGFP for exchange, one week post-nucleofection.

Equation 1.

Calculation of Cre exchange

$$\text{Percent Cre exchange} = \frac{(\text{EGFP positive} + \text{EGFP RFP double positive} + [\text{mKATE2 EGFP negative} - \text{starting mKATE2 negative}])}{100}$$

Equation 2.

Calculation of EGFP integration in mKATE2 negative cells

$$\text{Percent EGFP integration} = \frac{[\text{EGFP only positive}]}{[(\text{EGFP only positive} + \text{mKATE2 EFGP negative})]} \times 100\%$$

References

1. Araki, K., Araki, M., Yamamura, K., Site-directed integration of the cre gene mediated by Cre recombinase using a combination of mutant lox sites. Nucleic Acids Research, 30(19), e103 (2002).
2. Du, Z-W., Hu, B-Y., Ayala, M., Sauer, B., Zhang, S-C., Cre Recombination Mediated Cassette Exchange For Building Versatile Transgenic Human ESC lines. Stem cells (Dayton, Ohio), 27(5):1032-1041 (2009). doi:10.1002/stem.38.
3. Tas, H., Nguyen, C.T., Patel, R., Kim, N.H., Kuhlman, T.E., An Integrated System for Precise Genome Modification in *Escherichia coli*. Herman C, ed., PLoS ONE. 10(9), e0136963 (2015). doi:10.1371/journal.pone.0136963.

JB,TL,MAM 04/19-1

These products are covered by the Purchase Agreement as described in Exhibit 1.

EXHIBIT 1

PURCHASE AGREEMENT – LANDING PAD CELL LINES

This Product and its use are the subject of one or more of the following patents controlled by Sangamo BioSciences, Inc.: U.S. Patent Nos. 6,534,261, 6,607,882, 6,746,838, 6,794,136, 6,824,978, 6,866,997, 6,933,113, 6,979,539, 7,013,219, 7,030,215, 7,220,719, 7,241,573, 7,241,574, 7,585,849, 7,595,376, 6,903,185, 6,479,626, US20030232410, US20090203140 and corresponding foreign patent applications and patents.

BEFORE OPENING OR USING THIS PRODUCT, PLEASE READ THE TERMS AND CONDITIONS SET FORTH IN THIS PURCHASE AGREEMENT. YOUR USE OF THIS PRODUCT SHALL CONSTITUTE ACKNOWLEDGMENT AND ACCEPTANCE OF THESE TERMS AND CONDITIONS. If you do not agree to use this Product pursuant to the terms and conditions set out in this Purchase Agreement, please contact Sigma Technical Services within ten days of receipt to return the unused and unopened Product for a full refund; provided, however, that custom-made Products may not be returned for a refund.

The purchase of this Product conveys to you, the buyer, the non-transferable right to use the purchased Product for Licensed Research Use (see definition below) subject to the conditions set out in this Purchase Agreement. If you wish to use this Product for any purpose other than Licensed Research Use, you must first obtain an appropriate license (see information set out below).

This Product may not be used for any purpose other than Licensed Research Use. Product, as used herein, means the Cell Line and any derivative cells or cell lines created by the buyer which contain and/or incorporate genetic information derived from the Cell Line. Your right to use this Product for Licensed Research Use is subject to the following conditions and restrictions:

The type of license you have subject to this Agreement (Evaluation, Annual, Extended and Consumable) is listed on the outside of the package and/or on the invoice you received from us.

1. "Licensed Research Use" means any use for research purposes, other than:

(a) Licensing, selling, distributing, or otherwise providing the product or modified versions of it to any third party other than Sigma and its affiliates as provided herein: provided however, that you may provide the product or modified versions of it to researchers within your research organization located at the same research facility or campus who are similarly bound to the use restrictions herein;

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2. You may not transfer the Product, its components, or any materials made through the use of this Product, including further modified cells, to any third party without prior written approval of Sigma. Notwithstanding the foregoing, the Product or materials made through use of the Product may be transferred by you without such prior written approval to your legal affiliates or bona fide third party contractors performing paid work on your behalf, provided the use by such third party contractors is limited to performance of work for you and any results or product of such work shall not be shared by the third party contractor with any other person.

3. For Products covered by an "Evaluation" Purchase Agreement, this Agreement shall expire ninety (90) days following your receipt of the Product.

4. For Products covered by an "Annual" Purchase Agreement, this Agreement shall expire three hundred and sixty-five (365) days following your receipt of the Product.

5. For Products covered by an "Extended" Purchase Agreement, this Agreement shall remain in force in perpetuity following your receipt of the Product.

6. For Products covered by a “Consumable” Purchase Agreement, you may:

(a) Not expand, propagate, seed, bank, cryopreserve or store the product, its components, or any materials made through the use of this Product, outside of the original shipping device, including but not limited to vial, transwell plate and Petaka cell culture device. The Product can only be transferred and/or incorporated in an end-use assay. All Products that are not utilized in an end-use assay shall be immediately destroyed.

(b) Use Products for the provision of commercial services to a third party for monetary gain, but limited to only delivery of research results and data from use of the Product, and not the Product, its components, or any materials made through the use of this Product, including further modified cells.

7. Products covered by a "Consumable Vial" Purchase Agreement can only be incorporated in an end-use assay, seeded directly on the final assay plate (including, but not limited to 6-well, 12-well, 24-well, 48-well, or 96-well plates). All Products that are not utilized in an end-use assay plates shall be immediately destroyed.

8. Products delivered in a Petaka cell culture device can only be incorporated in an end-use assay for up to two (2) transwell plates (including, but not limited to 24-well or 96-well plates). All Products that are not utilized in an end-use assay for up to two (2) transwell plates shall be immediately destroyed.

9. Your right to use the Product will terminate immediately upon expiration of this Purchase Agreement, or in the event that you fail to comply with these terms and conditions. You shall, upon such termination of your rights, destroy all Product, any modified versions of the Product, and components thereof in your control, and notify Sigma of such in writing.
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