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

MCF10A RFP-TUBA1B
Breast Epithelial Cancer Cell Line with RFP-tagged TUBA1B

Catalog Number **CLL1039**
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 homologous recombination pathway was used to insert a transgene into a desired target location – the TUBA1B locus (NM_006082, α-tubulin isoform 1b). A donor construct containing a fluorescent reporter gene (RFP) flanked by sequences homologous to the target site was nucleofected into MCF10A cells along with ZFNs designed to cut near the genomic target site (see Figures 1a–c). Integration resulted in endogenous expression of the fluorescent fusion protein RFP–α-tubulin isoform 1b that polymerizes to form characteristic patterns of microtubules. Single cell knockin clones were isolated and analyzed (Figures 2a and 2b). A preferred clone was selected and carried for more than twenty passages to establish a stable cell line expressing RFP-TUBA1B from the endogenous genomic locus (Figure 2c).

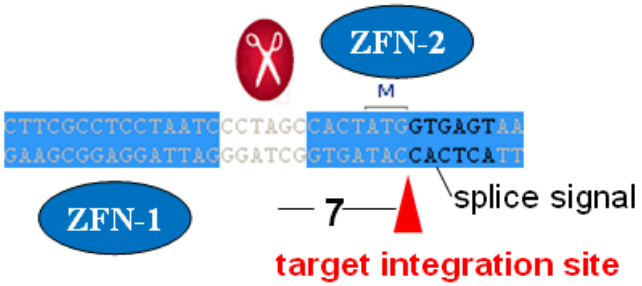
ZFN-mediated gene tagging in knockin cell lines provides the basis for the development of various assays for compound screening. Here, the target gene regulation and corresponding protein function are preserved in contrast to cell lines with overexpression under an exogenous promoter (Figure 2d). The RFP-TUBa1B cells are also shown to be sensitive to the mitotic inhibitor, Paclitaxel (Figure 2d).

For further information, go to the website: www.wherebiobegins.com/biocells

RFP and TagRFP are all synonymous for the same fluorescent reporter gene in this document. The RFP used in this cell line originated from Evrogen, referred to as TagRFP: http://evrogen.com/products/TagFPs.shtml

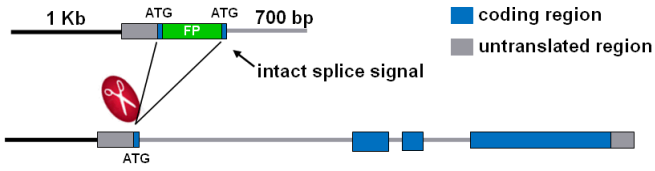
Figures 1a–1c.
The Design of Tag Sequence Integration at the TUBA1B Locus

Figure 1a.



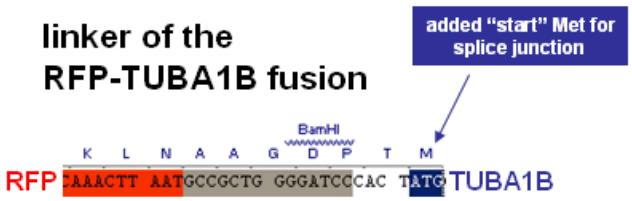
Schematic of the genomic sequence at the target region for integration of the fluorescent tag RFP DNA showing ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow).

Figure 1b.



Schematics of TUBA1B locus showing the coding region (blue), untranslated region (gray), and the ZFN cut site (scissors). The Donor (top) has the homology arms of indicated length and the FP sequence (green) fused to the beginning of TUBA1B coding sequence (the N-terminal fusion). The first exon contains ATG only. To preserve its splice signal, the FP sequence was inserted before the ATG. Another ATG was introduced in front of FP to initiate transcription.

Figure 1c.



Schematic of RFP-TUBA1B linker region. Bases shaded red are the 3′ end of RFP. Bases added for the creation of a linker are shaded gray. Unshaded and blue shaded bases are needed to create the proper splice junction. The amino acids encoded by the codons are shown in blue above the DNA sequence.

Cell Line Description
1 vial of modified MCF10A cells containing ~2×10^6 cells frozen in a cryoprotectant. Medium used is 1× Cell Freezing Medium-DMSO, Catalog No. C6164.

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.[1-3]

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 No. C8052 in sterile water. Store solution at 2–8 °C.
10.8 µL of EGF solution (1 mg/mL), prepared by dissolving Catalog No. 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 insure 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.

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.

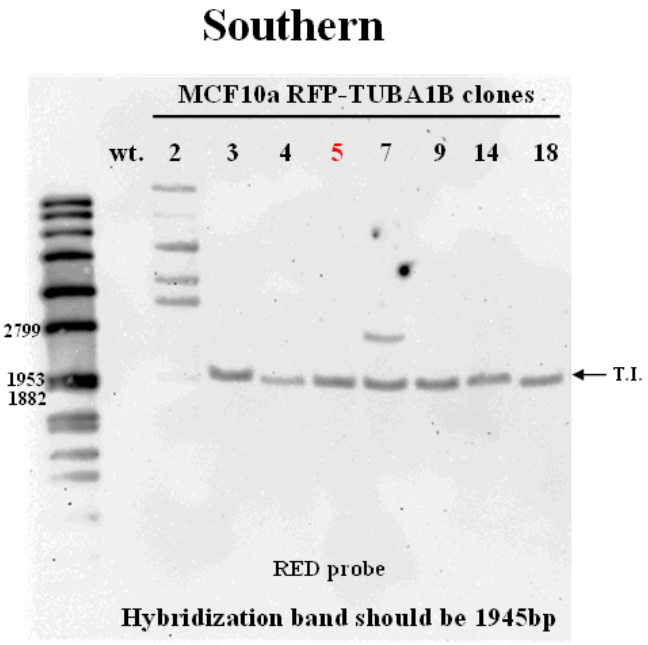
Subculturing Procedure
Volumes used in this procedure are for a 75 cm^2 flask; proportionally reduce or increase volume of dissociation medium for culture vessels of other sizes.

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 the flask and incubate at 37 °C for 15 minutes. This should remove the cells from the culture ware 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.[3]

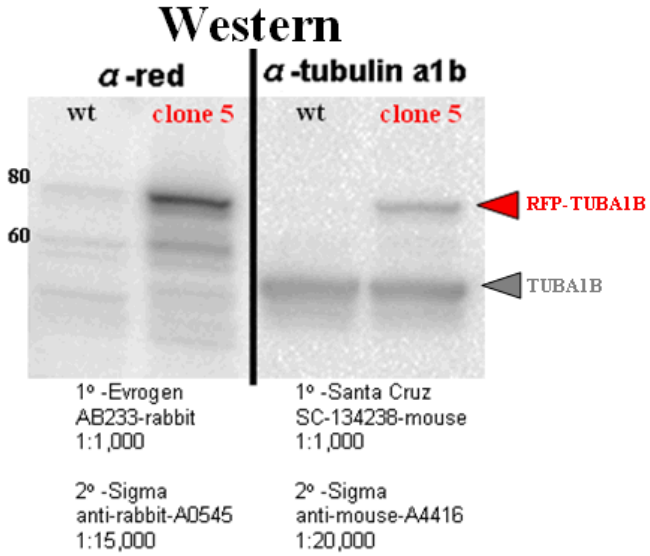
Results

Figure 2a.



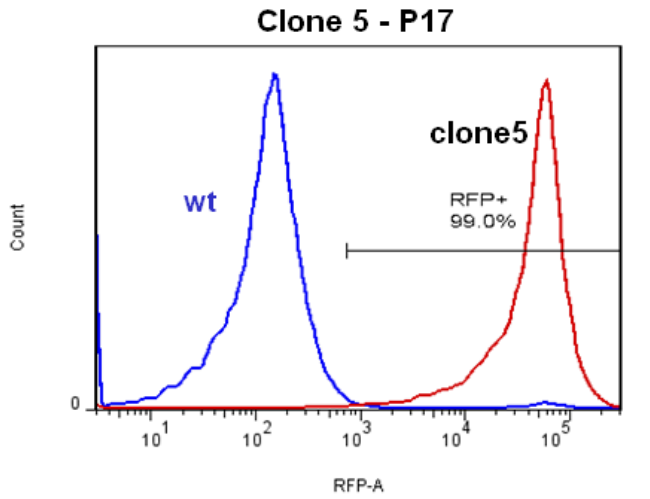
Plasmid donor was nucleofected into MCF10A cells to initially create the endogenous TUBA1B RFP tagged clones. Plasmid DNA can randomly integrate into the genome. To insure a clean clone, Southern analysis was performed. Southern blotting for TUBA1B single cell clones (lanes 3–10) with DIG-labeled RFP probe. With proper targeted insertion (T.I.) the *Pst* I digestion should yield a 1945 bp band in the clones. Random integration of the donor DNA (see Figure 1b.) is seen in clones 2 (lane 3) and 7 (lane 7). DIG labeled MW marker (lane 1) and negative control (parental line – wt, lane 2) are shown as well. Clone 5 (lane 6) was selected.

Figure 2b.



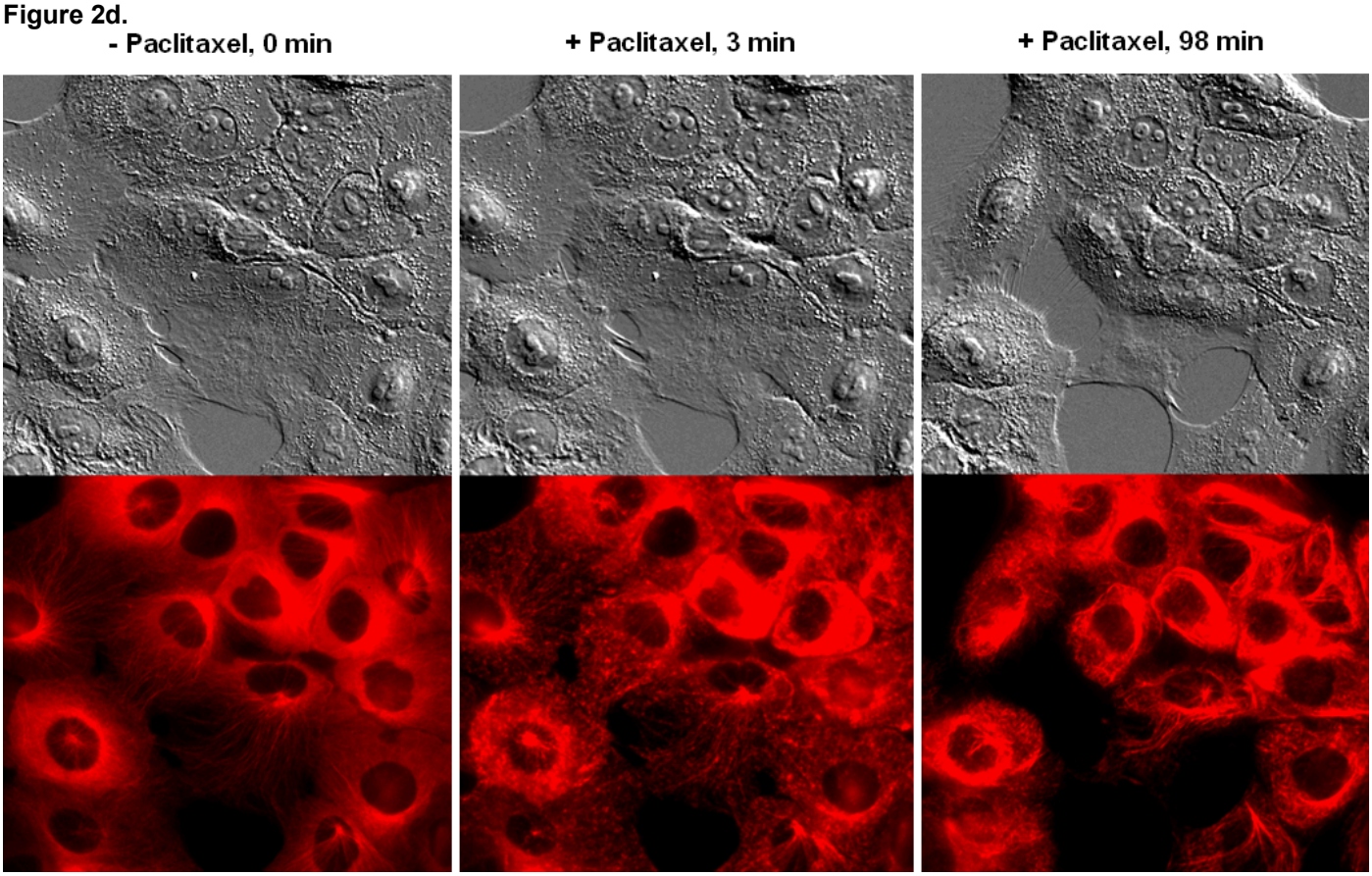
Western hybridizations were performed on total proteins isolated from wild type and RFP-TUBA1B MCF10A cells. Anti-TUBA1B indicates that both wild type and fusion proteins are produced in knockin cell lines. Anti-RFP detects the corresponding fluorescent fusion protein. The source and dilutions of the antibodies are indicated.

Figure 2c.



Flow cytometry analysis of RFP-TUBA1B single cell clone 5 at the end of viability test. 99% of the cells are RFP positive.

Figure 2d.



Paclitaxel effect on microtubules - Paclitaxel, a mitotic inhibitor used in cancer chemotherapy, is thought to stabilize microtubules and as a result, interfere with the normal breakdown of microtubules during cell division.[4] Catalog No. CLL1039 (RFP-tagged TUBA1B in MCF10A cells) was imaged by differential interference contrast (DIC) and fluorescence microscopy before and during treatment with 20 μM Paclitaxel (ex 530–560/em 590–650, 40×/1.4 oil). With increasing time, formation of typical tubulin bundles is observed. Tubulin polymerization is very sensitive to temperature and/or environment, and physiological conditions have to be maintained to observe the microtubules.

References

1. Centers for Disease Control, Biosafety in Microbiological and Biomedical Laboratories Human Health Service Publication No. (CDC) 21-1112. U.S. Dept. of Health and Human Services; 5th Edition (2009) U.S. Government Printing Office Washington D.C. The entire text is available online at www.cdc.gov/biosafety/publications/index.htm
2. Fleming, D.O., and Hunt, D.L., Biological Safety: Principles And Practices, 4th Edition, ASM Press, Washington, DC (2006).
3. Freshney, R.I., Culture of Animal Cells, a Manual of Basic Technique, 6th edition, published by John Wiley & Sons, Hoboken, NJ (2010).
4. Dumontet, C., and Jordan, M.A., Microtubule-binding agents: a dynamic field of cancer therapeutics. Nat. Rev. Drug Discov., **9**, 790-803 (2010).

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.sigma.com/biocells

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.

JF,NZ,ADM,AS,MAM 08/11-1