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

U2OS GFP-TUBA1B
Osteosarcoma Cell Line with GFP-tagged TUBA1B

Catalog Number **CLL1031**
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 (GFP) flanked by sequences homologous to the target site was nucleofected into U2OS cells along with ZFNs designed to cut near the genomic target site (see Figures 1a and 1b). Integration resulted in endogenous expression of fluorescent fusion protein GFP-TUBA1B that could polymerize 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 stable cell lines expressing GFP-TUBA1B from the endogenous genomic locus (Figures 2c and 2d).

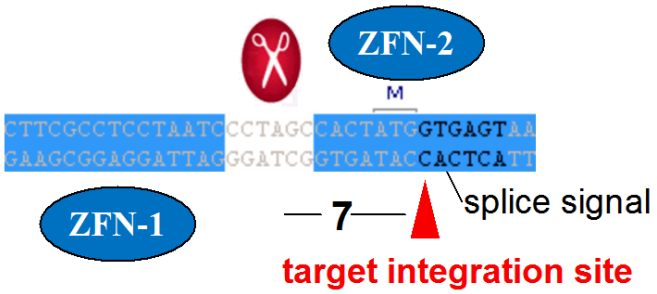
ZFN mediated gene tagging in knockin cell lines provides the basis for the development of various assays for compound screening. Here, the target gene regulation and corresponding protein function are preserved in contrast to cell lines with overexpression under an exogenous promoter (Figures 2e and 2f).

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

GFP, TagGFP2, and GFP2 are all synonymous for the fluorescent reporter gene in this document. The GFP used in this cell line originated from Evrogen, referred to as TagGFP2:
http://evrogen.com/products/TagFPs.shtml

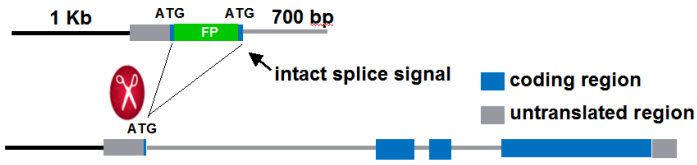
Figures 1a and 1b.
The Design of Tag Sequence Integration at the TUBA1B Locus

Figure 1a.



Schematic of the genomic sequence at the target region for integration of the fluorescent tag GFP 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.

Cell Line Description
1 vial of modified U2OS 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: osteosarcoma; bone

Age: 15 years

Gender: Female

Ethnicity: Caucasian

Morphology: Epithelial

Growth properties: Adherent

DNA profile
Short Tandem Repeat (STR) analysis:
Amelogenin: X
CSF1PO: 13
D13S317: 13
D16S539: 11,12
D5S818: 11
D7S820: 11,12
TH01: 6,9.3
TPOX: 11,12
vWA: 14,18

Parental Cell Line: ATCC® Catalog No. HTB-96™
Note: Please see HTB-96 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. 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: To make the complete growth medium, add fetal bovine serum, Catalog No. F2442, to a final concentration of 10% (v/v) in the base medium, McCoy's 5A Medium Modified, Catalog No. M9309. This medium is formulated for use with a 5% CO_2 in air atmosphere.

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 flask and incubate at 37 °C for 7 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.
   Subcultivation Ratio: 1:3 to 1:6
6. Incubate cultures at 37 °C.

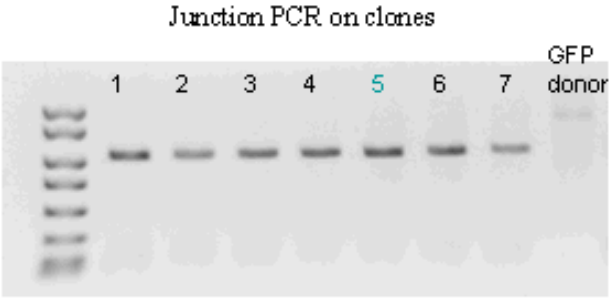
Note: More information on enzymatic dissociation and subculturing of cell lines is available in the literature.[3]

Results

Figures 2a–2f.

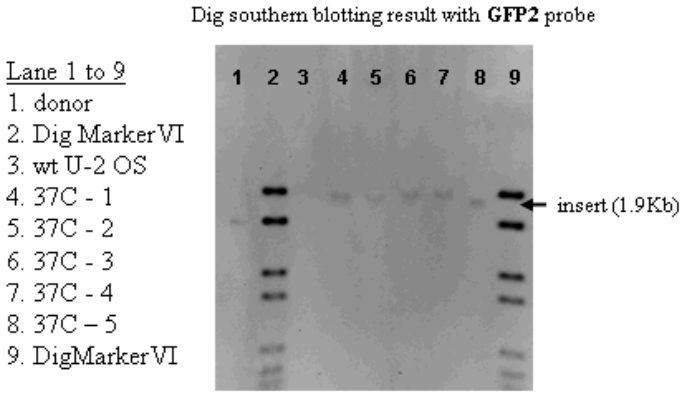
GFP-TUBA1B Single Cell Clone Verification

Figure 2a.



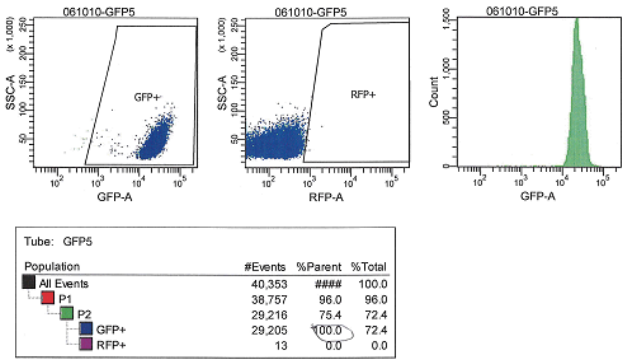
Gel electrophoresis analysis of a junction PCR of 7 single cell clones using the forward primer specific to the targeted GFP sequence and the reverse primer specific to the TUBA1B genomic sequence. Molecular size markers and the GFP-donor only control (cells were nucleofected without ZFNs) are also shown.

Figure 2b.



Southern blot shows no random integration from the plasmid donor initially used to create the clones. TUBA1B final single cell clones (lanes 4–8) with DIG-labeled GFP probe shows 1.9 kb band. Positive (donor plasmid, lane 1) and negative (parental line – wt, lane 3) controls are shown. Clone 37C-5 (lane 8) was chosen and became Catalog No. CLL1031.

Figure 2c.

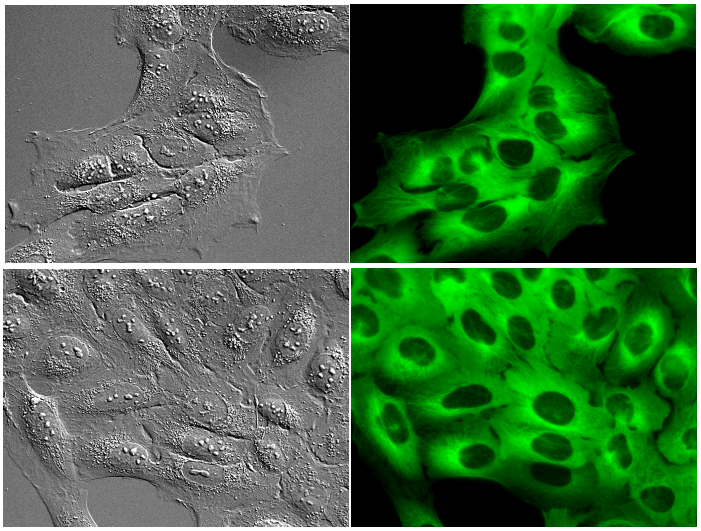


Flow cytometry analysis of GFP-TUBA1B single cell clone 37C-5 (Catalog No. CLL1031) at the end of viability test. 100% of the cells are GFP positive.

Figure 2d.

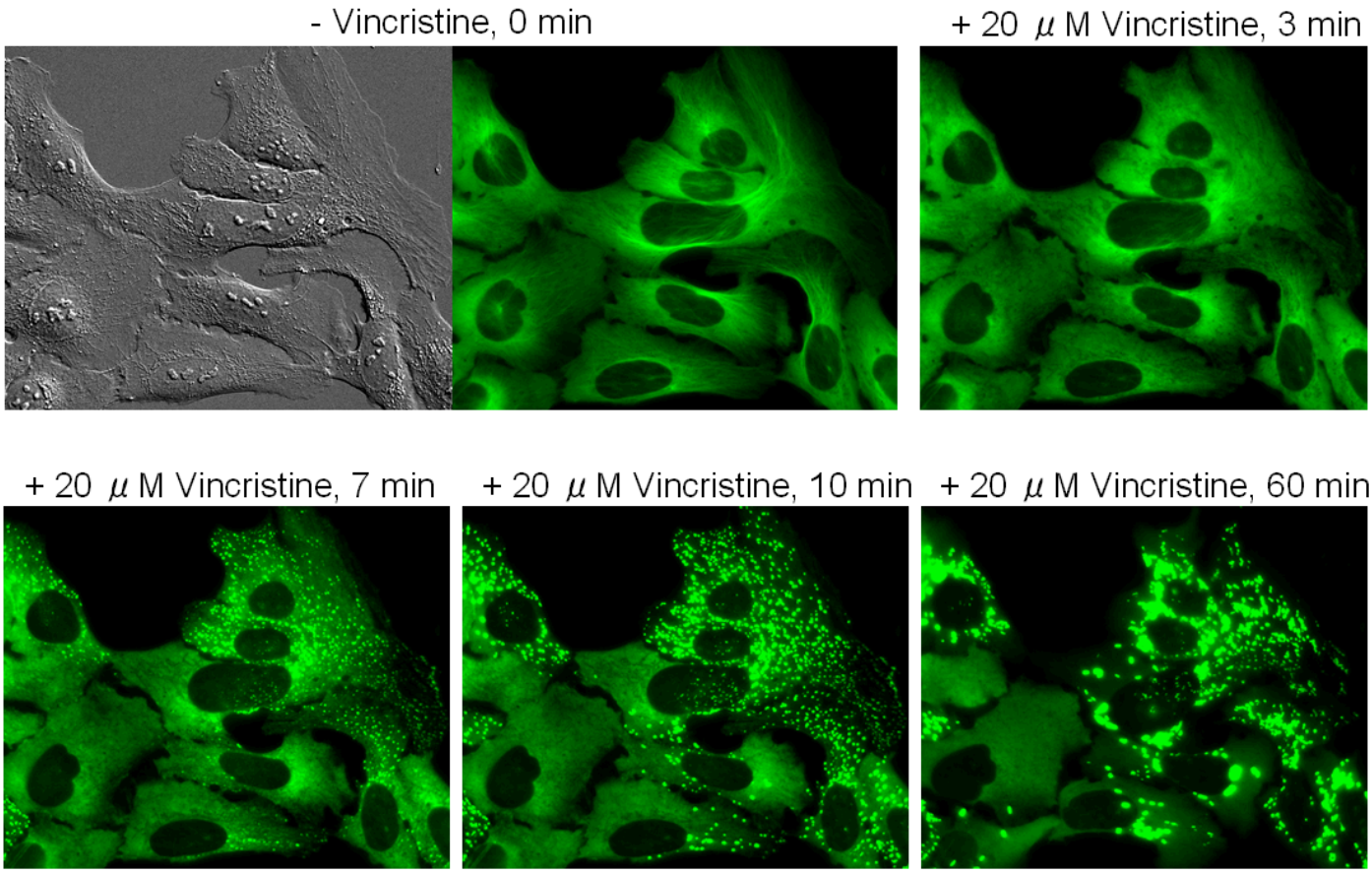
Western hybridizations were performed on total proteins isolated from wild type, GFP-TUBA1B (Catalog No. CLL1031), and RFP-TUBA1B (Catalog No. CLL1034) U2OS cells. Anti-TUBA1B indicates both wild type and fusion proteins are produced in knockin cell lines. Anti-GFP and Anti-RFP detect the corresponding fluorescent protein. The source and dilutions of the antibodies are indicated.

Figure 2e.



Differential interference contrast (DIC) and fluorescence microscopy images of an isolated cell clone (clone #5 from Figures 2a and 2b) expressing endogenous α-tubulin protein tagged with GFP (ex 450–490/ em 500–550, 40×/1.4 oil). Tubulin polymerization is very sensitive to temperature and/or environment. Physiological conditions have to be maintained to observe the microtubules.

Figure 2f.



Vincristine time course - Vincristine is a mitotic inhibitor used in cancer chemotherapy. Its mode of action is to bind to tubulin dimers, thereby, inhibiting the assembly of microtubule structures.[4] GFP tagged TUBA1B U2OS cells were exposed to 20 μM vincristine for sixty minutes. As time progresses, tubulin repolymerizes into a crystalline structure.

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. Lobert, S. et al., Interaction of vinca alkaloids with tubulin: A comparison of vinblastine, vincristine, and vinorelbine. Biochemistry, **35**, 6806-6814 (1996).

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

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.
HTB-96 is a trademark of American Type Culture Collection.

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