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Product Information

U2OS LMNB1-TUBA1B-ACTB
Osteosarcoma Cell Line with BFP-tagged LaminB1, GFP-tagged TUBA1B, and RFP-tagged ACTB

Catalog Number **CLL1218**
Storage Temperature –196 °C (liquid nitrogen)

Product Description

This product is a human U2OS cell line in which three genomic loci, LaminB1, TUBA1B, and ACTB have been endogenously tagged with fluorescent protein genes for Blue Fluorescent Protein (BFP), Green Fluorescent Protein (GFP), and Red Fluorescent Protein (RFP), respectively, using CompoZr® zinc finger nuclease (ZFN) technology. Imaging of the cell line shows fluorescent labeling of the three proteins that have normal cellular distribution patterns allowing this cell line to be used for high content screening to identify compounds that modulate cellular activity.

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 co-insert transgenes into three desired target locations: the LMNB1 locus (NM_005573, Lamin B1), the TUBA1B locus (NM_006082, α-tubulin isoform 1b), and the ACTB locus (NM_001101, β-actin).

For the LMNB-1 gene, a donor construct containing a fluorescent reporter gene (BFP) 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 Figure 1). Integration resulted in endogenous expression of fluorescent fusion protein BFP-laminB1 easily seen in the nuclear envelope (see Figure 2b).

For the TUBA1B gene, 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 Figure 1). Integration resulted in endogenous expression of fluorescent fusion protein GFP-α-tubulin that could polymerize to form characteristic patterns of microtubules (see Figure 2c).

For the ACTB gene, a donor construct containing a fluorescent reporter gene (RFP) 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 Figure 1). Integration resulted in endogenous expression of fluorescent fusion protein RFP-β-actin that could polymerize to form characteristic patterns of actin fibers (see Figure 2d).

Single cell knockin clones were isolated to establish a stable cell line co-expressing BFP-laminB1, GFP-α-tubulin and RFP-β-actin from the endogenous genomic loci (see Figure 2e). Flow cytometry showed a homogeneous population for each of the three fluorescent proteins (see Figure 3). Junction PCR showed at least one allele is tagged (see Figure 4a) and Southern analysis showed there were no off-target insertions of the FPs for the selected clone (see Figure 4b).

Data showing the effects of compound screening on fluorescently labeled tubulin or actin can be found in the technical bulletins for the following products: CLL1039 (paclitaxel on RFP-TUBA1B in MCF10A), CLL1031 (vincristine on GFP-TUBA1B in U2OS), and CLL1035 (cytochalasin B on RFP-ACTB in U2OS).

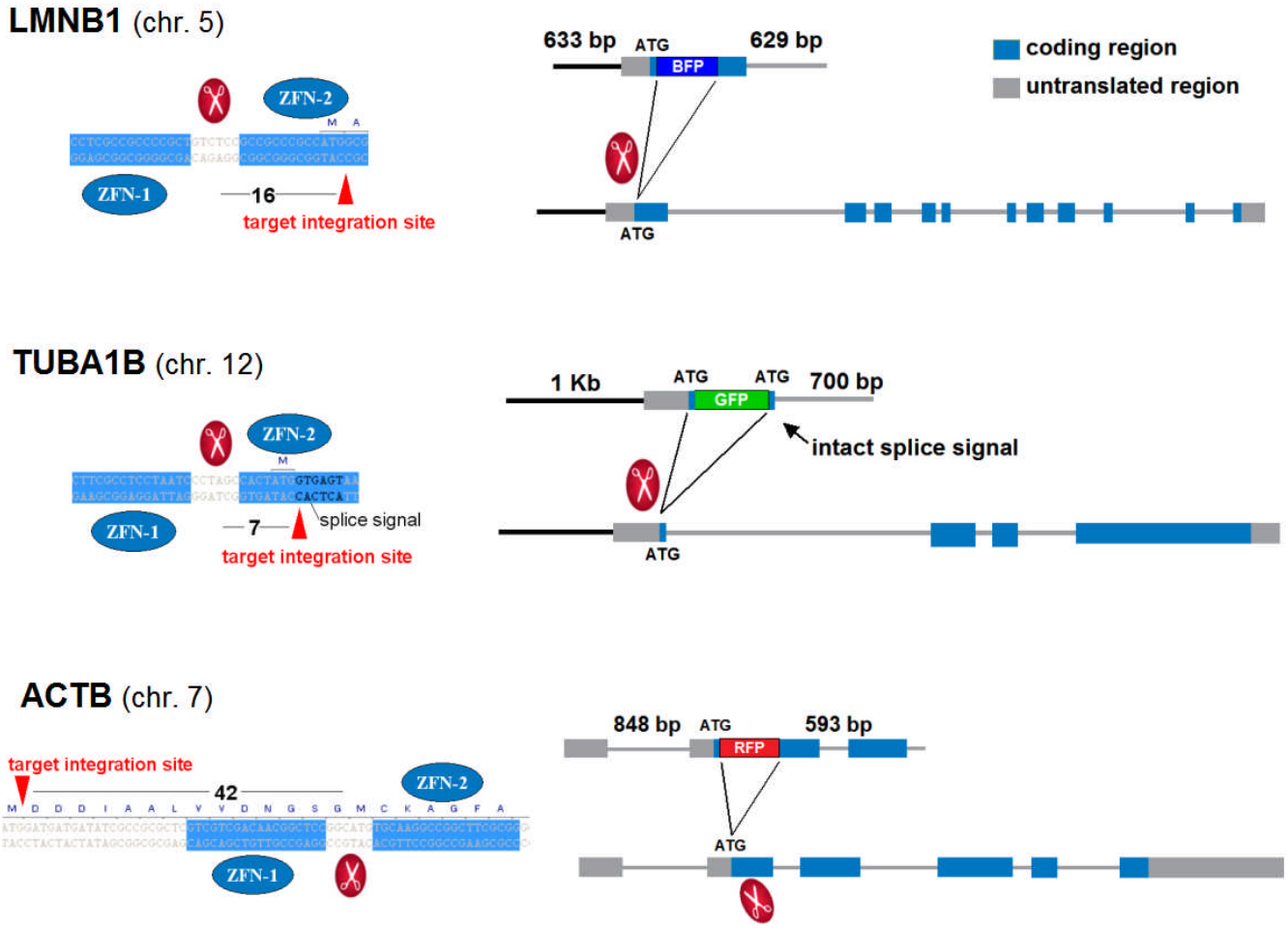
ZFN mediated gene tagging in knockin cell lines provides the basis for the development of various assays for compound screening. Here, the target gene regulation and corresponding protein function are preserved in contrast to cell lines with overexpression under an exogenous promoter.

BFP and TagBFP; GFP, TagGFP2, and GFP2; and RFP and TagRFP are synonymous for the fluorescent reporter genes in this document. The BFP, GFP, and RFP used in this cell line originated from Evrogen, referred to as TagBFP, TagGFP2, and TagRFP, respectively: http://evrogen.com/products/TagFPs.shtml

For further information on our CompoZr modified cell lines go to the website: www.sigma.com/biocells

Component		
U2OS cell line with BFP-tagged LaminB1, GFP-tagged TUBA1B, and RFP-tagged ACTB Catalog No. CLL1218	1 vial	One vial of modified U2OS cells contains ~2 × 10^6 cells in Cell Freezing Medium-DMSO 1×, Catalog No. C6164.

Figure 1.
The design of tag sequence integration at the LMNB1, TUBA1B, and ACTB Loci

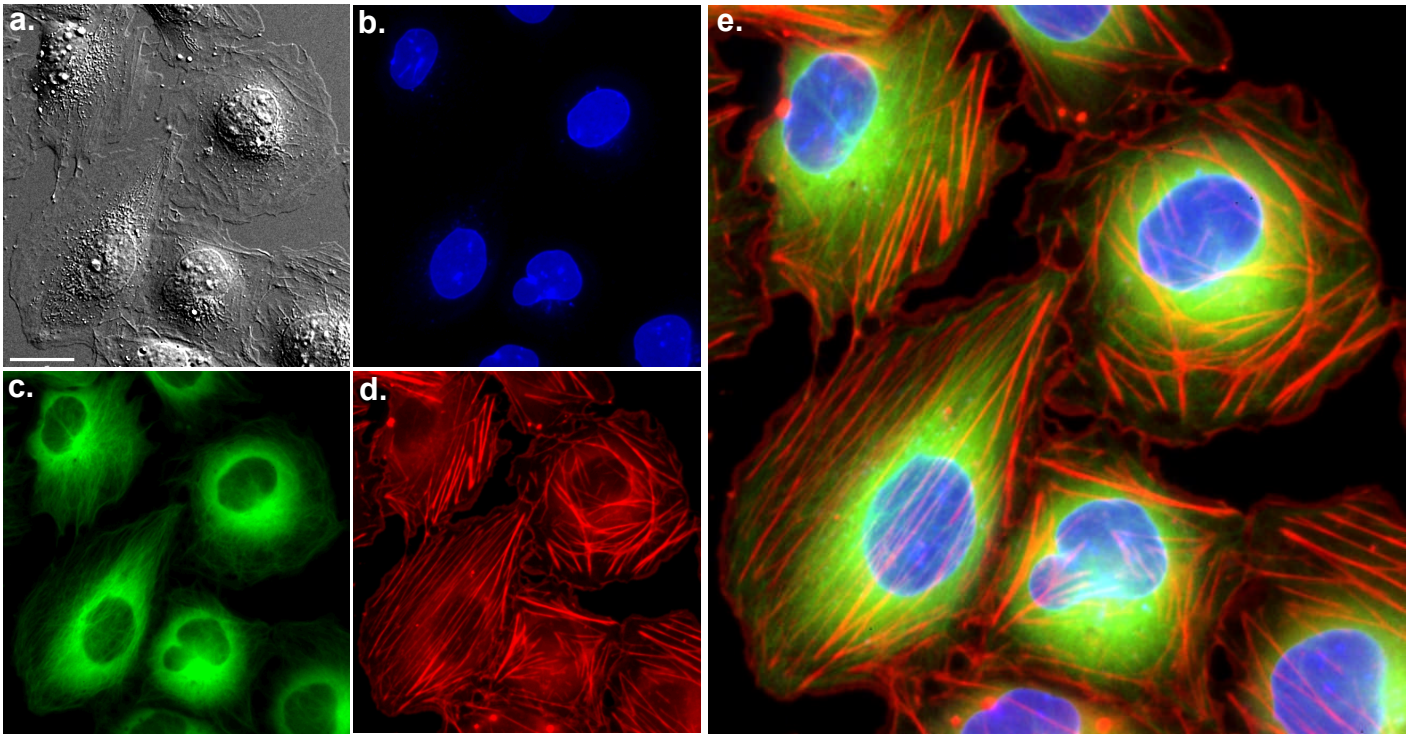


Left - Schematic of the genomic sequence at the target region for integration of the fluorescent tag - FP DNA showing ZFN binding sites (blue boxes), the ZFN cut site (scissors), and the tag sequence integration site (red arrow). Target locus names are indicated at the top with the chromosome number in the parentheses.

Right - Schematics of corresponding loci showing the coding region (blue), untranslated region (gray), and the ZFN cut site (scissors). The Donors (top) have the homology arms of indicated length and the FP sequence (boxed) fused to the beginning of the target gene coding sequence (all three inserts results in the N-terminal fusions).

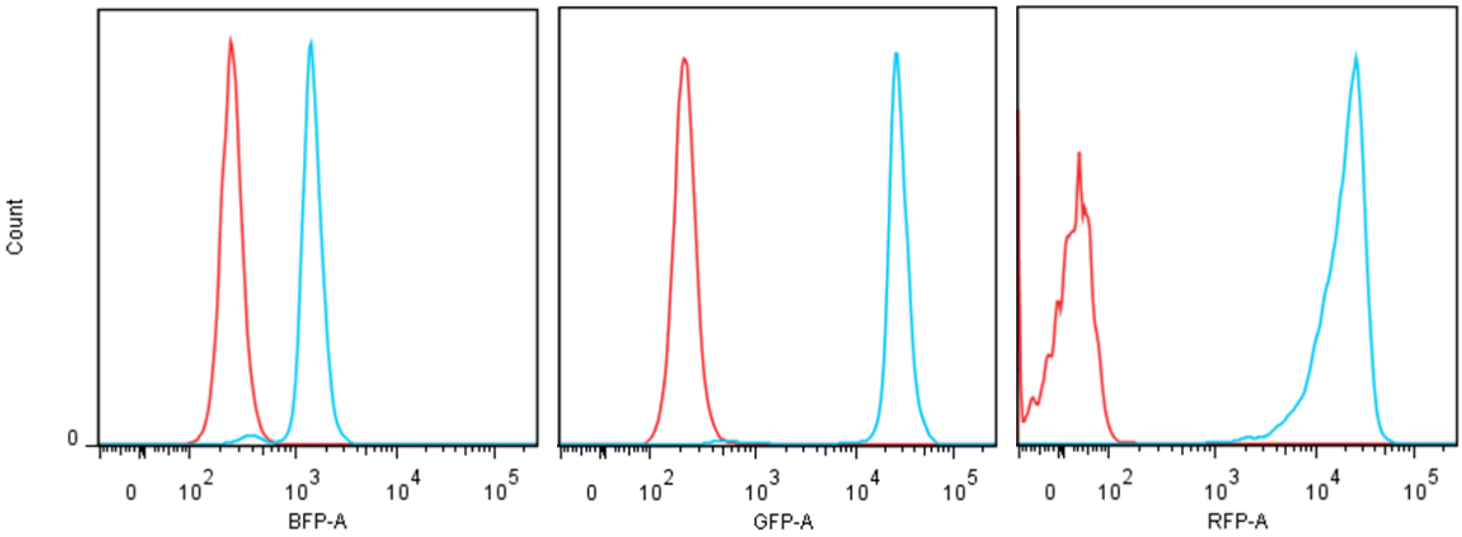
In the case of TUBA1B the first exon contains ATG only. To preserve its splice signal, the FP sequence was inserted before the ATG. Another ATG was introduced in front of FP to initiate transcription.

Figure 2.
Localization of endogenously tagged LMNB1 (BFP-laminB1), TUBA1B (GFP-α-tubulin), and ACTB (RFP-β-actin) in U2OS cells



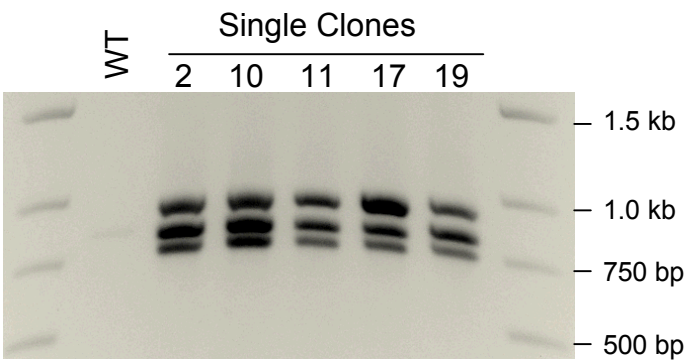
Three cytoskeletal genes were tagged by inserting a fluorescent reporter sequence behind the start codon of each locus. The integration resulted in endogenous expression of three distinct FP fusion proteins. The following loci were tagged: LMNB1 (lamin B1, nuclear envelope), TUBA1B (α-tubulin 1b, microtubules), ACTB (β-actin, actin stress fibers) by BFP, GFP, and RFP, respectively, within the same cells (U2OS osteosarcoma). Shown in this figure are the differential interference contrast (DIC) image (a.), fluorescence microscopy images of the BFP (b.), GFP (c.), and RFP (d.) channels and the corresponding overlay image (e.) of an isolated triple knockin clone. The cells were imaged live in Hanks' balanced salt solution (Catalog No. H8264) supplemented with 2% fetal bovine serum (Catalog No. F2442) using BFP filter set (ex 395-410/em 430-480), GFP filter set (ex 450–490/em 500–550), RFP filter set (ex 530–560/ em 590–650) and 40×/1.3 oil objective. The scale bar equals 25 μm.

Figure 3.
Expression levels of endogenously tagged LMNB1 (BFP-laminB1), TUBA1B (GFP-α-tubulin), and ACTB (RFP-β-actin) in U2OS cells



Flow cytometry analysis of BFP-LMNB1/GFP-TUBA1B/RFP-ACTB single cell clone #10 (blue) compared to the wild type U2OS (autofluorescence, red) using BD FACSAria™ III.

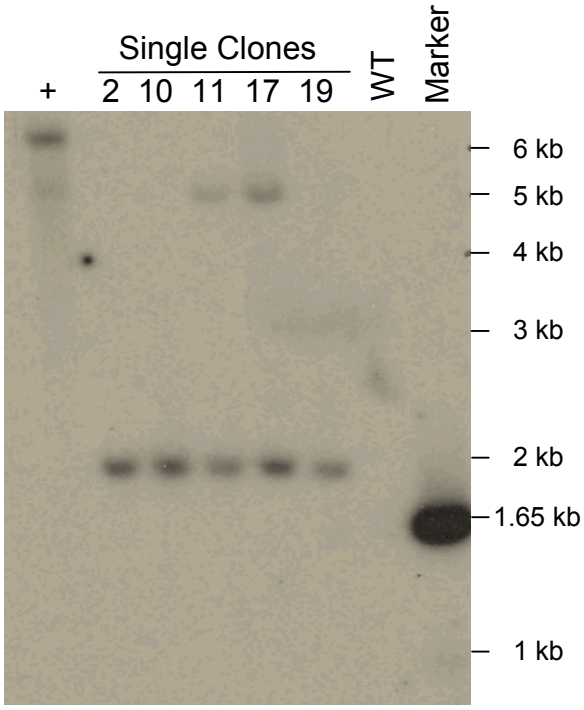
Figure 4a.
Molecular analysis to identify targeted integration in U2OS BFP-LMNB1/GFP-TUBA1B/RFP-ACTB clone



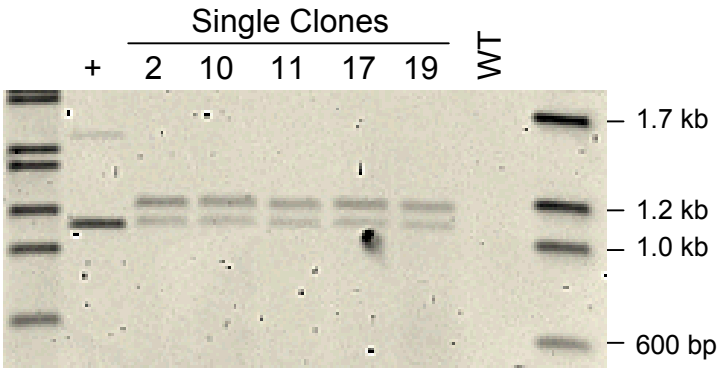
Multiplex junction PCR was performed on genomic DNAs isolated from the wild type U2OS (WT) and from triple-tagged single cell clones (a two-step PCR with annealing at 68 °C). The forward primers were specific to the BFP/GFP/RFP sequence and the reverse primers to the LMNB1/TUBA1B/ACTB genomic sequences. Expected fragments of 755 bp for LMNB1, 930 bp for TUBA1B, and 873 bp for ACTB confirm the targeted integration of BFP/GFP/RFP in front of the stop codons of LMNB1/TUBA1B/ACTB loci, respectively, for all clones tested. No PCR product can be detected in the wild type control. Junction PCR products were confirmed by sequencing.

Figure 4b.

Southern blotting allows clone selection with no random integration from the plasmid donors initially used for tagging. Genomic DNA from triple-tagged single cell clones and from U2OS wild type (WT - served as a negative control) was digested with restriction endonucleases *Pst* I (for detection of GFP off target insertion) or *Sty* I (for detection of BFP/RFP off target insertion).



Proper-targeted insertion of the GFP into the TUBA1B locus should produce a hybridized band of ~1.9 kb in size. Radioactive-labeled GFP probe was used. Positive (donor plasmid, +) and negative (parental line, WT) controls are shown as well.



The DIG-RFP probe hybridizes for both RFP and BFP (they are 95 % homologous). The expected hybridization band sizes are 1107 bp for BFP-LMNB1 and 1181 bp for RFP-ACTB. Positive (donor plasmid, +) and negative (parental line, WT) controls are shown as well. No random integration was detected. Clone #10 was chosen based on cell growth and morphology as well as molecular data. This clone became our final product CLL1218.

Cell Line Description
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

The STR profile of this cell line matches that of its 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. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses, or other adventitious 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: To make the complete growth medium, add fetal bovine serum, Catalog No. F2442, to a final concentration of 10% 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 vessel exploding or 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.

Complete Medium Preparation Instructions
To make the complete growth medium, add fetal bovine serum, Catalog No. F2442, to a final concentration of 10% 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.

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]

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. & 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).

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 website at www.wherebiobegins.com/biocells

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