3AB-OS Human Osteosarcoma Cancer Stem Cell Line

Cancer Cell Line

Cat. # SCC141

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

Certificate of Analysis

Background

Osteosarcoma is the most common type of bone cancer in children and young adults and is characterized by the presence of osteoid (bone formation) within the tumor. Increasing evidence suggests that a small population of cancer-initiating cells (CICs) or cancer stem cells (CSCs) are responsible for tumorigenesis and drug resistance to anti-cancer therapies.

The 3AB-OS human osteosarcoma cancer stem cell line is derived from long-term treatment of MG-63 human osteosarcoma cells with 3-aminobenzamide (3AB), a potent inhibitor of poly (ADP-ribose) polymerase. In the parental MG-63 cells, brief treatment with 3AB resulted in osteocyte differentiation. However prolonged treatment (~100 days) with 3AB induced osteocyte death and selective enrichment of a heterogeneous stable population with cancer stem cell-like characteristics[1].

3AB-OS contains three different cell morphologies: (1) spindle-shaped, and (2) polygonal-shaped fibroblastoid cells, and single phase-bright rounded-shaped cells. 3AB-OS expresses a number of pluripotent stem cell markers (Oct3/4, hTERT, nucleostemin, Nanog) along with genes for inhibition of apoptosis (HIF-1α, FLIP-L, Bcl-2, XIAP, IAPs, and survivin)[1]. 3AB-OS is a useful tool for investigating the mechanisms by which CSCs may originate. 3AB-OS may also be used as a model system for therapeutic interventions against human osteosarcoma.

Short Tandem Repeat (STR) Profile

D3S1358: 15, 17	D16S539: 10, 11
TH01: 9.3	CSF1PO: 10, 11
D21S11: 28, 29	Penta D: 11, 13
D18S51: 13, 18	vWA: 18
Penta E: 12	D8S1179: 11, 14, 15
D5S818: 11	TPOX: 9
D13S317: 13	FGA: 20, 21
D7S820: 8, 12	Amelogenin: X

Tumor cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Storage & Handling

3AB-OS Human Osteosarcoma Cancer Stem Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥ 1X10^6 viable cells.
- Cells are tested negative for HPV-16, HPV-18, Hepatitis A, C, Herpesvirus type 6, 7, 8 and HIV-1 & 2 viruses by PCR.
- Cells are negative for mycoplasma contamination.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Representative Data

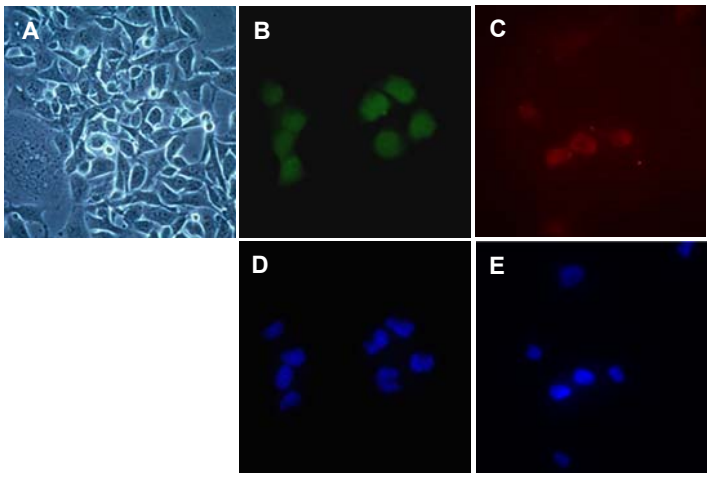

Figure 1: Day 1 after thaw (**A**). 3AB-OS cells express cancer stem cell markers Nanog (**B**) and Oct-4 (**C**). Cells were counterstained with Dapi (**D, E**).

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

Please visit www.millipore.com for additional product information and references.
Submit your published journal article, and earn credit toward future purchases. Visit www.millipore.com/publicationrewards to learn more!

Rev. 1.0/2017-05-26/SCC141/VC

Protocols

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   Cells are thawed and expanded in DMEM-High Glucose (Sigma Cat. No. D6546), 10% FBS (EMD Millipore Cat. No. ES-009-B), 2 mM L-Glutamine (EMD Millipore Cat. No. TMS-002-C) and 1X Penicillin-Streptomycin Solution (EMD Millipore Cat. No. TMS-AB2-C).
2. Remove the vial of frozen 3AB-OS cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of 3AB-OS Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of 3AB-OS Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.
12. The next day, exchange the medium with 10-15 mL of fresh 3AB-OS Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 85-90% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use. Typical split ratio is 1:6.

Subculturing Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the 85-90% confluent layer of 3AB-OS cells.
2. Rinse the T75 flask twice with 10 mL 1X PBS. Aspirate after each rinse.
3. Apply 3-5 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 8 mL of 3AB-OS Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2 mL of 3AB-OS Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of Cells

3AB-OS Human Osteosarcoma Cancer Stem Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

References

1. Di Fiore R, Santulli A, Ferrante RD, Giuliano M, De Blasio A, Messina C, Pirozzi G, Tirino V, Tesoriere G, Vento R. (2009) Identification and expansion of human osteosarcoma-cancer-stem cells by long-term 3-aminobenzamide treatment. *J Cell Physiol* 219 (2): 301-313.
2. Di Fiore R, Fanale D, Drago-Ferrante R, Chiaradonna F, Giuliano M, De Blasio A, Amodeo V, Corsini LR, Basan V, Tesoriere G, Vento R, Russo A (2013) Genetic and molecular characterization of the human osteosarcoma 3AB-OS cancer stem cell line: a possible model for studying osteosarcoma origin and stemness. *J Cell Physiol* 228(6): 1189-1201.
3. Di Fiore R, Drago-Ferrante R, D'Anneo A, De Blasio A, Santulli A, Messina C, Carlisi D, Tesoriere G, Vento R. (2013) Differentiation of human osteosarcoma 3AB-OS stem-like cells in derivatives of the three primary germ layers as a useful in vitro model to develop several purposes. *Stem Cell Discovery* 13(3): 118-201.

ACADEMIC USE AGREEMENT
(subject to local law)

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL MILLIPORESIGMA ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means 3AB-OS Human Osteosarcoma Cancer Stem Cell Line (SCC141)

"Academic Research" means any internal *in vitro* research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.