Data Sheet

U2OS EGFP-H2B Human Osteosarcoma Cell Line

Cancer Cell Line

SCC293

Pack size: ≥ $1x10^6$ viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Chromosomal abnormalities underlie a substantial proportion of cancers, including the majority of solid tumors.[1] Chromosome instability manifests in a range of defects, from aneuploidy (abnormal number of entire chromosomes) to chromosomal breakage and rearrangements.[1] Understanding the mechanisms of chromosomal instability is key to unlocking the multifaceted roles that they play in cancer etiology and progression.

The U2OS osteosarcoma is one of the first and most widely used cancer cell lines, especially for the study of mitosis, apoptosis, and the cellular mechanisms that give rise to cancer. U2OS cells undergo chromosome breakage and chromothripsis at high frequency.[2] The expression of EGFP fused to a histone marker in the U2OS EGFP-H2B cell line allows for visualization of chromosome dynamics by light microscopy, a powerful tool in unraveling the complex interactions and molecular players involved in chromosomal instability.[3] Expression of EGFP-H2B in this cell line is maintained via selection with blasticidin. The U2OS EGFP-H2B cell line facilitates expanded insights to chromosomal biology, allowing immediate relevancy to the many studies using the well-characterized U2OS background.

Source

The U2OS EGFP-H2B Human Osteosarcoma cell line was derived from U2OS cells stably transfected with a construct expressing EGFP-histone-H2B.[3] The parental U2OS cell line was derived from an osteosarcoma from a 15-year-old female.[2]

Short Tandem Repeat (STR Profile)

D3S1358:	16	D13S317:	13
D7S820:	11, 12	D16S539:	11, 12
vWA:	14, 18	TH01:	6, 9.3
FGA:	20	TPOX:	11, 12
D8S1179:	12, 14	CSF1PO:	12, 13
D21S11:	31	Amelogenin:	X
D18S51:	12, 14	Penta D:	9
D5S818:	8, 11	Penta E:	10, 13

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

Quality Control Testing

- U2OS EGFP-H2B human osteosarcoma cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

BG-1 human ovarian adenocarcinoma cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

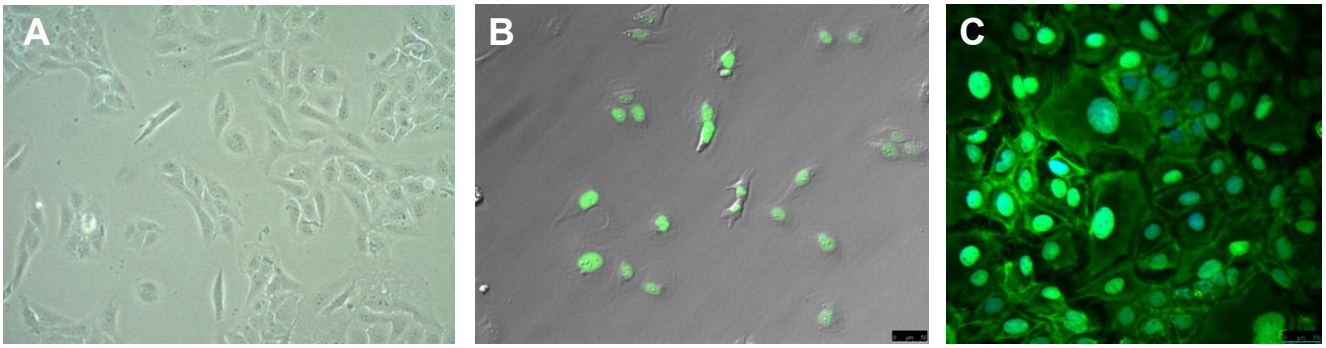

Figure 1. Bright-field image of U2OS EGFP-H2B human osteosarcoma cells two days (A) after thaw. Cells express EGFP-H2B (B) and actin (C, Cat. No. P5282).

Protocols

Thawing the Cells

Catalogue numbers in parenthesis can be purchased from SigmaAldrich.com unless otherwise noted.

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

   Cells are thawed and expanded in U2OS EGFP-H2B Expansion Medium comprising DMEM-High Glucose medium (D6429), 2 mM L-Glutamine (G7513), 10 mM HEPES (H0887), 10% FBS (ES-009-B), and 5 mg/mL blasticidin (203350) for selection.

2. Remove the vial of frozen U2OS EGFP-H2B 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 U2OS EGFP-H2B 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 15 mL of U2OS EGFP-H2B 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.

Subculturing the Cells

1. Do not allow the cells to grow to confluency. U2OS EGFP-H2B cells should be passaged at ~ 70-80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of U2OS EGFP-H2B cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase® and incubate in a 37 °C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of U2OS EGFP-H2B Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of U2OS EGFP-H2B Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

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

Cyropreservation of the Cells

U2OS EGFP-H2B human osteosarcoma cells may be frozen in U2OS EGFP-H2B Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Thompson SL, Compton DA. *Chormosome Res.* 2011; 19(3):433-444.
2. Pontén J, Saksela E. *Int J Cancer.* 1967; 2(5):434-447.
3. Wang H et al. *Oncotarget* 2017; 8(30):48671-48687.

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