U266B1-Fluc-Puro/eGFP-Neo

Product Description

Product Name: U266B1-Fluc-Puro/eGFP-Neo
Catalog Number: CL176
Lot Number: IMP027

Species: Human (*Homo sapiens*)
Tissue: Peripheral blood
Disease: Myeloma
Parental cells: U266B1 (ATCC® TIB-196™)*
Morphology: Lymphoblast
Growth mode: Suspension
Reporter genes: Firefly luciferase (Fluc)
Enhanced green fluorescent protein (eGFP)
Selection genes: Puromycin (Puro)
Neomycin (Neo)

This is a cell line derived from the U266B1 cell line (ATCC® TIB-196™). Parental U266B1 cells were transduced with, 1) LV-SFFV-Fluc-P2A-Puro (Imanis #LV012) encoding the firefly luciferase (Fluc) cDNA under the spleen focus-forming virus (SFFV) promoter and linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide, and 2) LV-SFFV-eGFP-P2A-Neo (Imanis #LV067) encoding the enhanced green fluorescent protein (eGFP) cDNA under the spleen focus-forming virus (SFFV) promoter and linked to the neomycin resistance gene (Neo) via a P2A cleavage peptide. High Fluc- and eGFP- expressing populations were generated by selection using puromycin and G418, followed by selection with a methylcellulose-based semi-solid medium. The lentiviral vectors are self-inactivating (SIN) vectors in which the viral enhancer and promoter have been deleted. Transcription inactivation of the LTR in the SIN provirus increases biosafety by preventing mobilization by replication competent viruses and enables regulated expression of the genes from the internal promoters without *cis*-acting effects of the LTR[1].

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Mycoplasma Testing

This cell line has been tested for mycoplasma contamination and is mycoplasma free.

Recommended Uses

These cells are suitable for *in vitro* and *in vivo* experimentation.

The Fluc transgene facilitates *in vivo* noninvasive bioluminescent imaging of implanted cells. eGFP is not recommended for whole animal in-live imaging. Rather, samples can be collected post-mortem for analysis by conventional fluorescence microscopy or flow cytometry.

References

[1]Miyoshi et al. J Virol. 1998. 72:8150-8157.

Biosafety Notice

This cell line was generated by transduction with a lentiviral vector. Cell lines transduced with lentiviral vectors are classified as biosafety level 2 reagents and should be used under appropriate biosafety level for institutional guidelines.

Storage Instructions

Remove cells from the dry ice packaging and immediately store in the vapor phase above liquid nitrogen (below -130ºC).

Complete Growth Medium

ATCC Formulated RPMI-1640 Medium
15% Fetal Bovine Serum (FBS)
1% Penicillin/Streptomycin
3 µg/mL Puromycin
1 mg/mL G418

Puromycin and G418 should NOT be added to the medium until a culture has been well established from the thawed cells (about 1 week). It is also recommended that a backup frozen cell stock be generated (see below) before adding puromycin and G418 to the growth medium.

Caution! Typical commercial puromycin stocks are provided at a concentration of 10 mg/mL or 10,000X.

Thawing Instructions

1. Thaw cells by gently swirling in a 37ºC water bath. To limit contamination, do not submerge the O-ring and cap.
2. When cells are ~70% thawed (about 1 min), remove the vial and wipe down with 70% ethanol. Allow tube to dry completely.
3. In a biosafety cabinet, transfer the cells into a 15 mL conical tube containing 5 mL of complete growth medium. Centrifuge cells at ~300 x *g* for 4-5 min.
4. Remove supernatant and resuspend cells in complete growth medium to a final density of 1 x 10^6 cells/mL. Transfer the cells to a T25 or T75 suspension culture flask.
5. Incubate the culture at 37°C with 5% CO_2.

Note: Cell viability will decrease 1-2 days after thaw, but will begin to recover within 4-5 days.

Subculturing Instructions

Passage cells by dilution in fresh complete growth medium. If desired, use centrifugation to remove excess debris as follows:

1. Pipet the cell suspension gently to dislodge any cells loosely attached to the culture flask. Transfer the desired volume (half, one-fourth, etc.) of the cells to a conical tube.
2. Centrifuge at ~150 x g for 3 min. (Note: a short, low speed spin is recommended to limit the amount of cell debris in the pellet.)
3. Remove the supernatant and resuspend the cells in complete growth medium. Transfer to an appropriately sized flask.

The cells should be subcultured as needed to maintain a density between 5 x 10^5 and 2 x 10^6 cells/mL. A complete media change is recommended approximately every 4 days for optimal cell viability.

Freezing Medium

These cells can be amplified and used to generate additional frozen stocks. Cryopreservation of low passage stocks is recommended. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium without puromycin and G418 supplemented with 5-10% DMSO.

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Certificate of Analysis

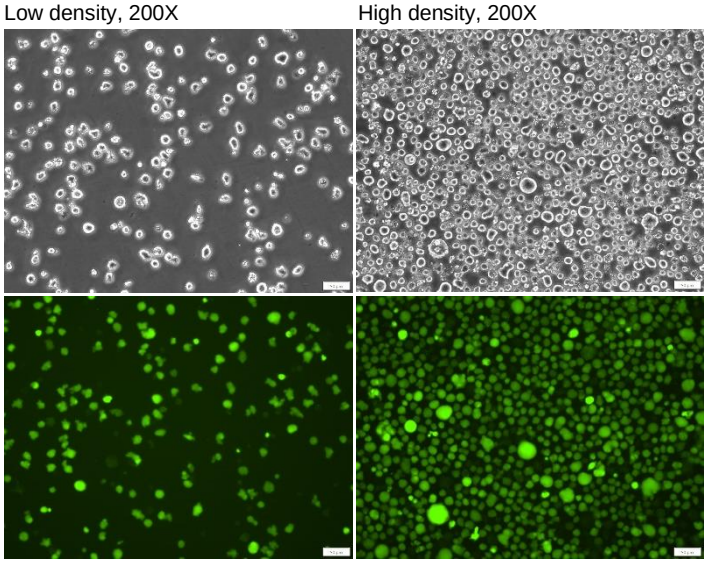
Testing performed by Imanis Life Sciences

Test Description	Results
Post thaw viable cell recovery	86%
Viable cells per vial	~ 1.2 x 10^7
Sterility	No contamination detected
Mycoplasma	No contamination detected
Luciferase expression	Pass QC
Fluorescence expression	Pass QC
Average doubling time	52.7 hours*

*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

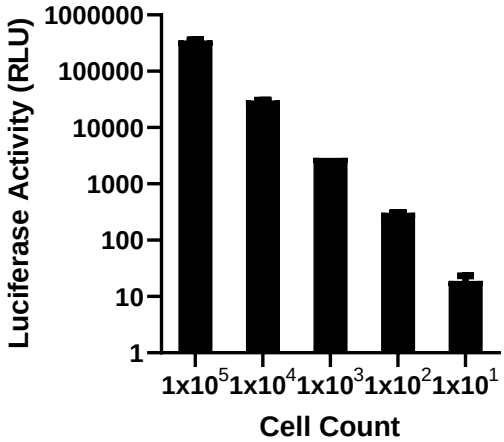
Morphology

Low density, 200X High density, 200X



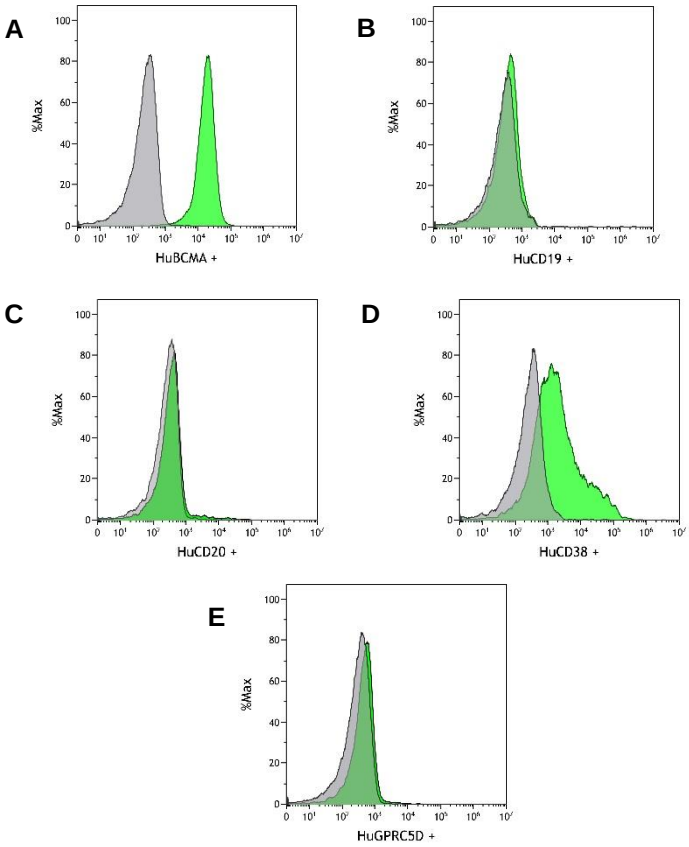
Low- and high- density photos taken at various times after thawing.

Luciferase Expression



The indicated number of cells were placed in wells of a 96-well plate. After the addition of 15 mg/mL d-luciferin, bioluminescence was immediately read using a microplate reader.

Expression Profiling of Surface Markers



U266B1-Fluc-Puro/eGFP-Neo cells were stained with isotype control antibody (grey) or an anti-HuBCMA antibody (A), anti-HuCD19 antibody (B), anti-HuCD20 antibody (C), anti-HuCD38 antibody (D), or anti-HuGPRC5D antibody (E) and analyzed by flow cytometry.

Quality Control by: AWD
Quality Assurance by: RLV
Effective Date: 15-Dec-2023

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