U266B1-Fluc-Puro

Product Description

Product Name: U266B1-Fluc-Puro
Catalog Number: CL173
Lot Number: IMP021

Species: Human (*Homo sapiens*)
Tissue: Peripheral blood
Disease: Myeloma
Parental cells: U266B1 (ATCC® TIB-196™)*
Morphology: Lymphoblast
Growth mode: Suspension
Reporter gene: Firefly luciferase (Fluc)
Selection gene: Puromycin (Puro)

This is a cell line derived from the U266B1 cell line (ATCC® TIB-196™). Parental U266B1 cells were transduced with 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. A high Fluc-expressing population was generated by selection using puromycin followed by selection with a methylcellulose-based semi-solid medium. The lentiviral vector is a self-inactivating (SIN) vector 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].

* The ATCC trademark and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection

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 luciferase transgene facilitates non-invasive *in vivo* bioluminescence imaging.

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

Puromycin 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 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.

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.

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 supplemented with 5-10% DMSO.

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Certificate of Analysis

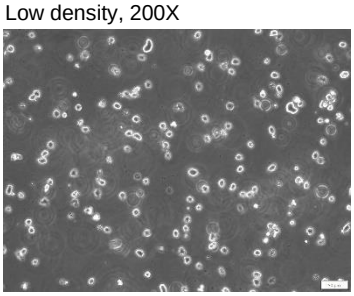
Testing performed by Imanis Life Sciences

Test Description	Results
Post thaw viable cell recovery	88%
Viable cells per vial	~ 8 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Luciferase expression	Pass QC
Average doubling time	82.3*

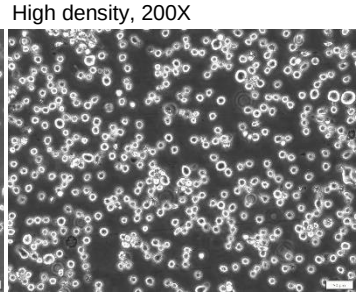
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology

Low density, 200X

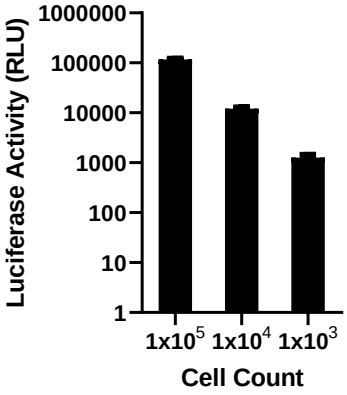


High density, 200X



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

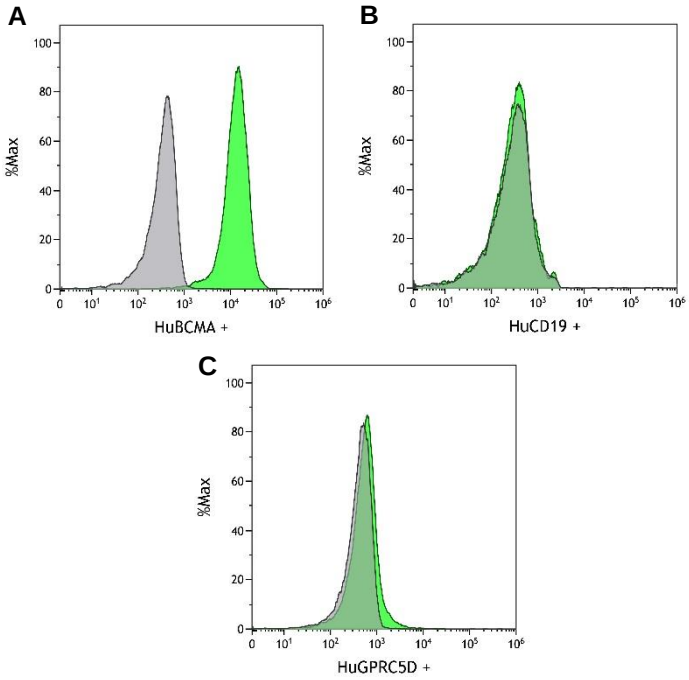
Luciferase Expression



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.

Quality Control by: AWD
Quality Assurance by: RLV
Effective Date: 08-Jun-2023

Expression Profiling of Surface Markers



U266B1-Fluc-Puro (green) or isotype control (Parental U266B1; grey) cells were stained with an anti-HuBCMA antibody (A), anti-HuCD19 antibody (B), or anti-HuGPRC5D antibody (C) and analyzed by flow cytometry.

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