Daudi-Fluc-Puro

Product Description

Product Name: Daudi-Fluc-Puro
Catalog Number: CL160
Lot Number: CL-IM194

Species: Human (*Homo sapiens*)
Tissues: Peripheral blood
Cell type: B Lymphoblast
Parental cells: Daudi (ATCC® CCL-213™)*
Morphology: Lymphoblast
Growth mode: Suspension
Reporter gene: Firefly luciferase (Fluc)
Resistance gene: Puromycin (Puro)

This is a cell line derived from the human B-lymphoblast Daudi cell line (ATCC® CCL-213™). Parental Daudi cells were transduced with LV-SFFV-Luc2-P2A-Puro (Imanis #LV012) encoding 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. Selection was carried out using puromycin and 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 tested negative for mycoplasma contamination.

Recommended Uses

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

The luciferase transgene facilitates *in vivo* noninvasive bioluminescent imaging of implanted cells.

References

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

Storage Instructions

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

Complete Growth Medium

RPMI-1640 Medium (RPMI)
10 mM HEPES
1 mM sodium pyruvate
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin

5 µg/mL of puromycin may be added to the growth medium. 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

Thaw cells by gently swirling in a 37ºC water bath. To limit contamination, do not submerge the O-ring and cap.
When cells are ~70% thawed (~1 min), remove the vial and wipe down with 70% ethanol. Allow tube to dry completely.
In a biosafety cabinet, transfer the cells into a 15 mL conical tube containing 5 mL of complete growth medium. Centrifuge cells at ~200 x *g* for 3-5 min.
Remove supernatant and resuspend cells in 1 mL complete growth medium. Remove an aliquot for counting.
Dilute the cells further with growth medium to achieve a final density of 1 x 10^6 cells/mL. Transfer the cells to a T25 or T75 flask based on volume.
Incubate the culture at 37°C with 5% CO_2.

Subculturing Instructions

The cells should be subcultured as needed to maintain a density between 5 x 10^5 and 2 x 10^6 cells/mL. The cells can be passaged by dilution in fresh complete growth medium. Regular passage using centrifugation as described below is recommended to limit the amount of debris in cultures.

Pipet the cell suspension gently to dislodge any cells loosely attached to the culture flask. Transfer the desired volume of the cells to a conical tube.
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.)
Remove supernatant and resuspend cells in complete growth medium. Transfer to an appropriate sized flask.

Freezing Medium

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

Notes:

This cell line is positive for EBV.

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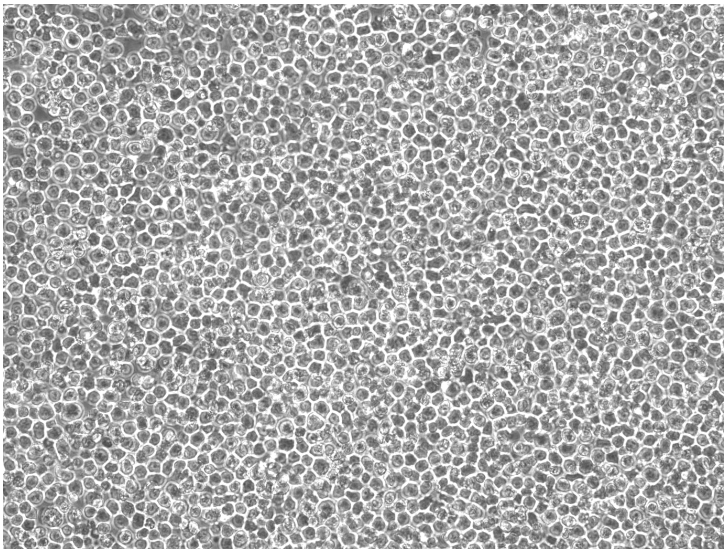
Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	95%
Cells per vial	~1.6 x 10^7
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin expression	Pass QC
Luciferase resistance	Pass QC
Average doubling time	39 h*

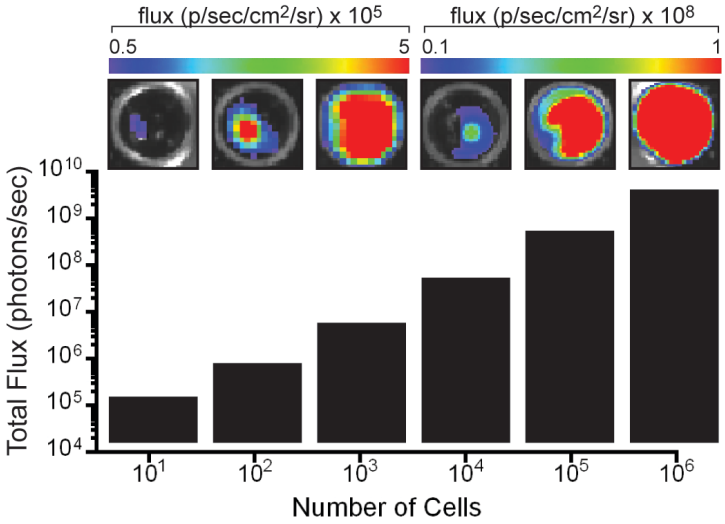
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology



Cell photo taken at 200X magnification.

Luciferase Expression



The indicated number of cells were placed in wells of a 96-well plate. After the addition of 3 mg/mL d-luciferin, the plate was immediately imaged using an IVIS Spectrum. The total flux (photons/sec) was plotted as a function of cell number.

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