C1498-Fluc-Neo/eGFP-Puro

Product Description

Product Name: C1498-Fluc-Neo/eGFP-Puro
Catalog Number: CL145
Lot Number: CL-IM156

Species: Mouse (*Mus musculus*)
Strain: C57BL/6J
Cell type: Lymphoblast (acute myeloid leukemia; AML)
Parental cells: C1498 (ATCC® TIB-49™)*
Morphology: Lymphoblast
Growth mode: Suspension
Reporter genes: Firefly luciferase (Fluc)
Enhanced green fluorescent protein (eGFP)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

This is a cell line derived from the murine acute myeloid leukemia C1498 cell line (ATCC® TIB-49™). Parental C1498 cells were transduced with 1) LV-Fluc-P2A-Neo (Imanis #LV011) encoding the firefly luciferase (Fluc) cDNA under the spleen focus-forming virus (SFFV) promoter and linked to the neomycin resistance gene (Neo) via a P2A cleavage peptide, and 2) LV-eGFP-PGK-Puro (Imanis #LV031) encoding the enhanced green fluorescent protein (eGFP) cDNA under the SFFV promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter. A high Fluc- and eGFP-expressing population was generated by selection using G418 and puromycin followed by selection using 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 certified mycoplasma free.

Cell Line Authentication

The parental C1498 cell line was authenticated and certified free of interspecies cross contamination by STR profiling.

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. Fluc and eGFP are immunogenic and may cause tumor rejection in immunocompetent mice. For the most consistent results, immunocompromised mice are recommended for studies.

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 -130°C).

Complete Growth Medium

High glucose DMEM
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
1 mg/mL G418
1 µg/mL puromycin

G418 and 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 G418 and 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 37°C water bath. To limit contamination, do not submerge the O-ring and cap.
2. When cells are ~70% thawed (less than 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 ~200 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium. Remove an aliquot for counting.
5. Dilute the cells further with growth medium to achieve a final density between 1 and 2 x 10^6 cells/mL. Transfer the cells to a T25 or T75 flask based on volume.
6. 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. Occasional passage using centrifugation as described below is recommended to limit the amount of debris in cultures.

1. Pipet the cell suspension gently to dislodge any cells loosely attached to the culture flask. Transfer the desired volume (half, one fifth, 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 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 antibiotics supplemented with 5-10% DMSO.

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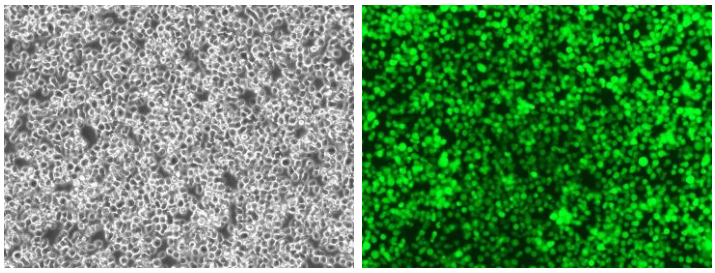
Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	86%
Cells per vial	~ 9 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC
Average doubling time	20.1 h*

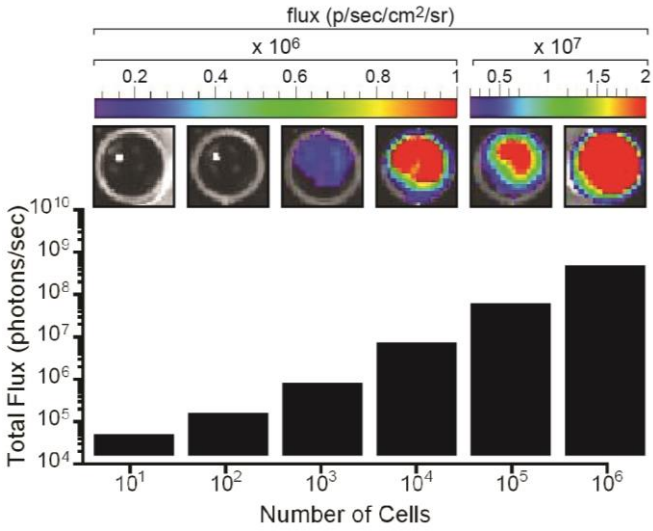
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology



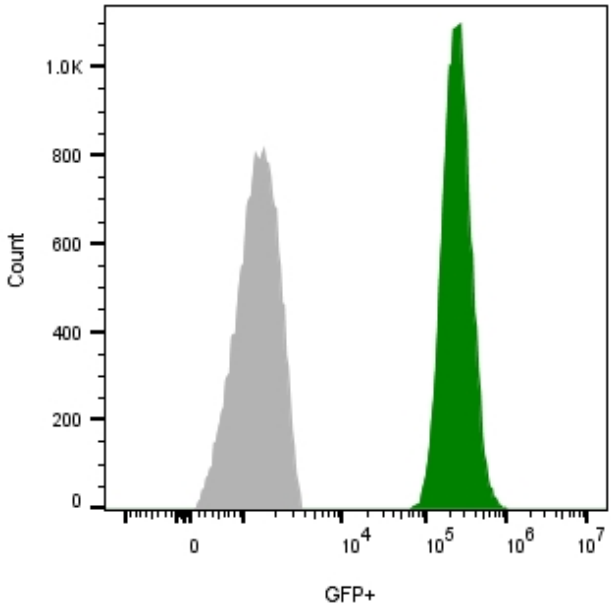
Cell photos taken at 200x magnification.

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.

Fluorescence Expression



C1498-Fluc-Neo/eGFP-Puro (green) or isotype control (C1498-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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