Nalm6-Fluc-Neo/eGFP-Puro

Product Description

Product Name: Nalm6-Fluc-Neo/eGFP-Puro
Catalog Number: CL150
Lot Number: IMP038

Species: Human (*Homo sapiens*)
Tissues: Peripheral blood
Cell type: Lymphoma
Parental cells: Nalm6 (ATCC® CRL-3273™)*
Morphology: Lymphocyte-like
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 human B cell precursor leukemia Nalm6 cell line (ATCC® CRL-3273™). Parental Nalm6 cells were transduced with 1) LV-Luc2-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 semi-solid methyl-cellulose-based 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 (Miyoshi et al. J Virol. 1998. 72:8150-8157).

* 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.

Cell Line Authentication

Authentication of the parental Nalm6 cell line was confirmed 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 postmortem for analysis by conventional fluorescence microscopy or flow cytometry.

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

RPMI-1640 Medium (RPMI) supplemented with 10 mM HEPES
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 37ºC 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 ~300 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 of 1 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. Cells should reach full confluency 4 days after thawing.

Subculturing Instructions

The cells should be subcultured through dilution in fresh complete growth medium as needed to maintain a density between 3 x 10^5 and 3 x 10^6 cells/mL. As needed passage using centrifugation as described below can be used 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 appropriately 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

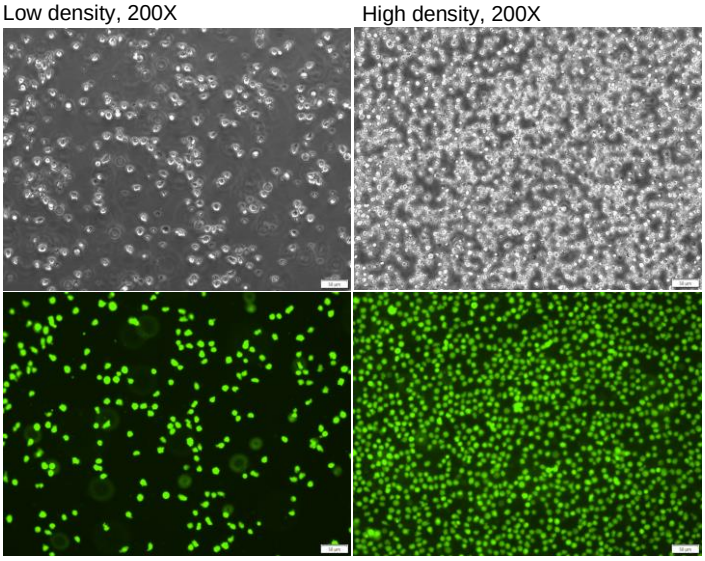
Test description	Result
Post thaw viable cell recovery	81%
Cells per vial	~ 9.5 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC
Average doubling time	21.2 h*

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

Morphology

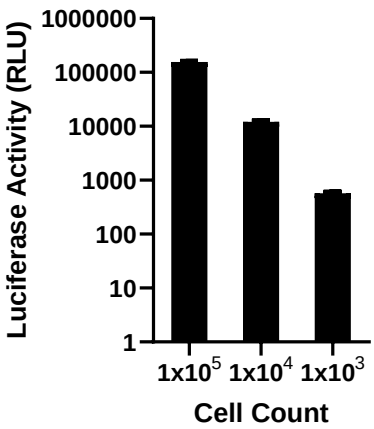
Low density, 200X

High density, 200X



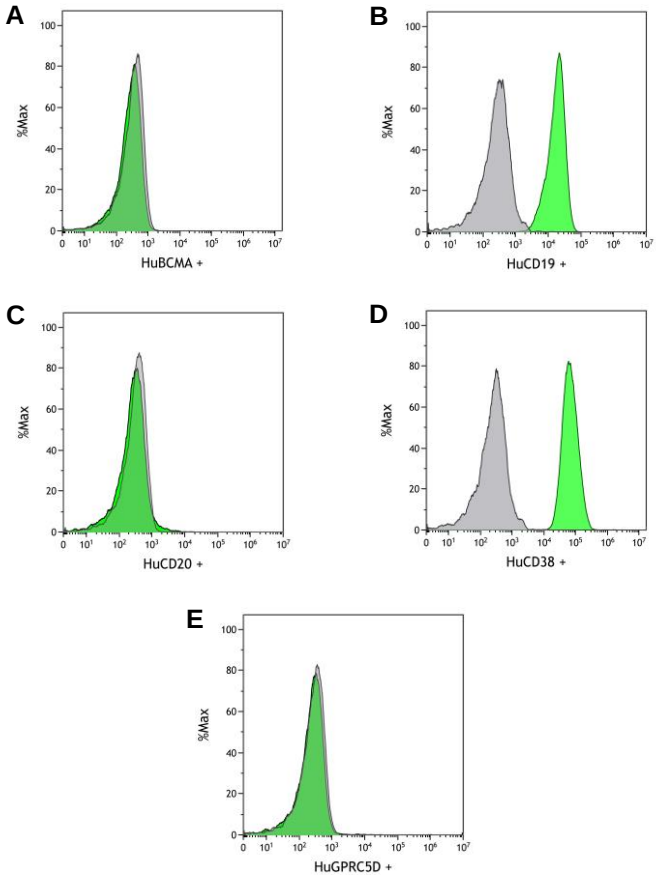
Low- and high-density photos taken at different times after thawing.

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



Nalm6-Fluc-Neo/eGFP-Puro were stained with isotyped control antibody (grey) or marker specific (green) anti-HuBCMA (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: 16-Apr-2024

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