Nalm6-Fluc-hNIS

Product Description

Product Name: Nalm6-Fluc-hNIS
Catalog Number: CL143
Lot Number: CL-IM152

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)
Human sodium iodide symporter (hNIS)

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 LV-Luc2-P2A-hNIS (Imanis #LV023) encoding the firefly luciferase (Fluc) cDNA under the spleen focus-forming virus (SFFV) promoter and linked to the human sodium iodide symporter (hNIS) cDNA via a P2A cleavage peptide. A high Fluc and hNIS expressing population was generated by two rounds of selection using 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 certified mycoplasma free.

Cell Line Authentication

The parental Nalm6 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 and hNIS transgenes facilitate *in vivo* noninvasive bioluminescent and high resolution 3D SPECT/PET imaging, respectively, 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) containing 10 mM HEPES
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin

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 ~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 3 x 10^6 cells/mL (do NOT use more than 20 mL total). 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

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.

The cells should be subcultured as needed to maintain a density between 3 x 10^5 and 3 x 10^6 cells/mL. The cells can be passaged by dilution in fresh complete growth medium (without centrifugation). However, regular passage using centrifugation as described above is recommended to limit the amount of debris in cultures.

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

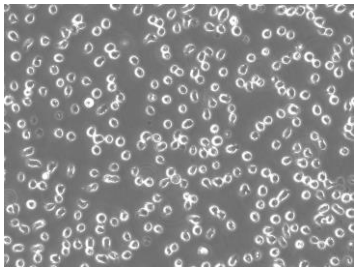
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	96%
Cells per vial	~ 1.4 x 10^7
Sterility	No contamination detected
Mycoplasma	No contamination detected
Luciferase expression	Pass QC
NIS expression	Pass QC
Average doubling time	24.2 h*

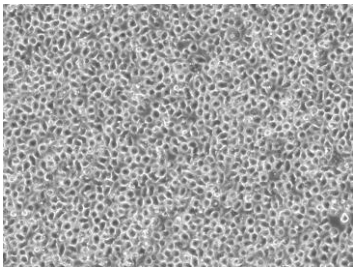
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology

Low density, 200X

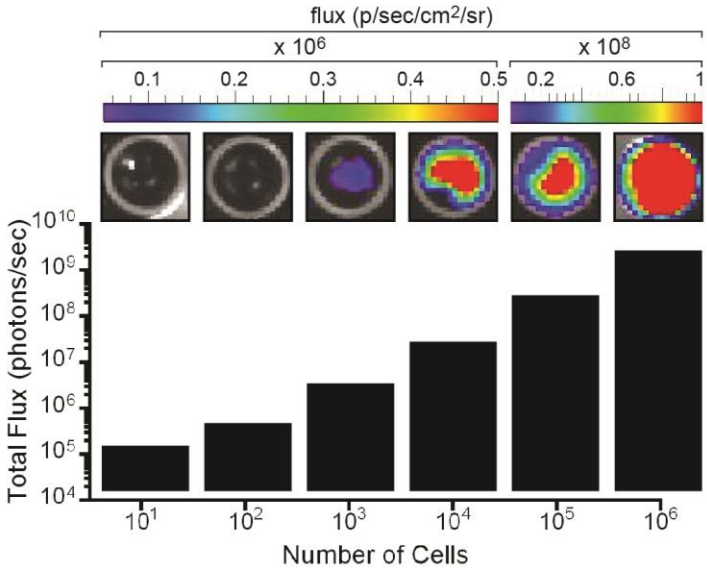


High density, 200X



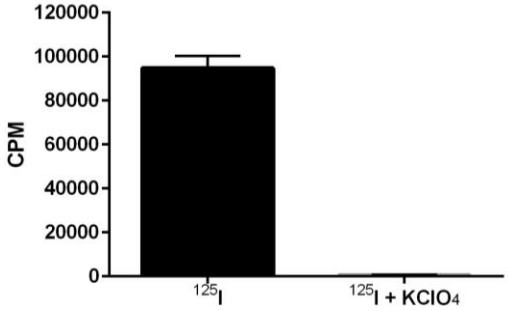
Low and high density photos taken at various times after thawing.

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.

NIS Expression



Uptake of ^{125}I by 3 x 10^5 cells was assayed in the presence or absence of $KClO_4$, an inhibitor of NIS-mediated ^{125}I uptake.

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