A375-Fluc-Neo/hNIS-Puro

Product Description

Product Name: A375-Fluc-Neo/hNIS-Puro
Catalog Number: CL087
Lot Number: CL-IM84

Species: Human (*Homo sapiens*)
Tissue: Skin
Cell type: Malignant melanoma
Parental cells: A375 (ATCC® CRL-1619™)
Morphology: Epithelial
Growth mode: Adherent
Reporter genes: Firefly luciferase (Fluc)
Human sodium iodide symporter (hNIS)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

This is a polyclonal population derived from the malignant melanoma A375 cell line (ATCC® CRL-1619™). Parental A375 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 linked to the neomycin resistance gene (Neo) via a P2A cleavage peptide, and 2) LV-hNIS-P2A-Puro (Imanis #LV019) encoding the humans iodide symporter (hNIS) cDNA under the SFFV promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High Fluc and hNIS expressing cells were selected using G418 and puromycin. 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].

Mycoplasma Testing

The A375-Fluc-Neo/hNIS-Puro cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

In light of studies suggesting that 18-36% of cell lines utilized in biomedical research are contaminated or completely misidentified,[2,3] several funding organizations, including NIH, as well as major publishers, including those affiliated with the American Associate for Cancer Research (AACR), require cell lines used in research to be authenticated prior to publication[4,5]. The parental A375 cell line used to generate A375-Fluc-Neo/hNIS-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

Recommended Uses

A375-Fluc-Neo/hNIS-Puro cells are suitable for *in vitro* and *in vivo* experimentation. A375 cells form tumors post implantation into immunosuppressed mice[6]. The Fluc and hNIS transgenes facilitate *in vivo* noninvasive bioluminescent and high-resolution 3D PET/SPECT imaging, respectively, of implanted cells.

References

[1]Miyoshi et al. J Virol. 1998. 72:8150-8157.
[2]Hughes et al. BioTechniques 2007. 43: 575-586.
[3]Chatterjee et al. Science 2007. 315:928-931.
[4]https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html
[5]http://www.aacrjournals.org/site/InstrAuthors/ifora.xhtml#celllineuse
[6]Gershwin et al. J Natl Cancer Inst. 1977. 58:1455-1461.

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

Dulbecco's Modified Eagle's Medium (DMEM)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
0.6 mg/mL G418 (to maintain high Fluc expression)
1 µg/mL puromycin (to maintain high hNIS expression)

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 pre-warmed complete growth medium without selection drugs. Centrifuge cells at ~250 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium without selection drugs. Transfer cells to a T75 flask containing 10 mL pre-warmed complete growth medium without selection drugs.
5. Incubate the culture at 37°C with 5% CO_2. After 48 hours, replace the culture supernatant with complete growth medium containing 0.6 mg/mL G418 and 1 µg/mL puromycin. Cells should reach full confluency 3-4 days after thawing.

Subculturing Instructions

Volumes are given for a T75 flask; increase or decrease as needed. To maintain high Fluc and hNIS expression, it is recommended that cells be subcultured in the presence of 0.6 mg/mL G418 and 1 µg/mL puromycin. A375-Fluc-Neo/hNIS-Puro cells should be passaged when they reach 90-100% confluency.

1. Remove culture medium from cells.
2. Carefully wash the cell monolayer with 5-10 mL of phosphate buffered saline.
3. Add 2 mL of 0.25% Trypsin-EDTA solution to the flask and incubate at 37°C until cells have dissociated (approx. 2-5 min).
4. Neutralize the trypsin by adding 8 mL complete growth medium, and mix by gently pipetting up and down.
5. Transfer desired portion of the cells to a fresh T75 flask. Add fresh complete growth medium to a total volume of 10 mL and return cells to 37°C/5% CO_2 incubator.

For maintenance a subcultivation ratio of 1:10 is recommended. At this ratio cells will be ready for passage every 3-4 days.

Freezing Medium

A375-Fluc-Neo/hNIS-Puro cells can be amplified and used to generate additional frozen stocks. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium without selection drugs supplemented with 5-10% DMSO.

A375-Fluc-Neo/hNIS-Puro

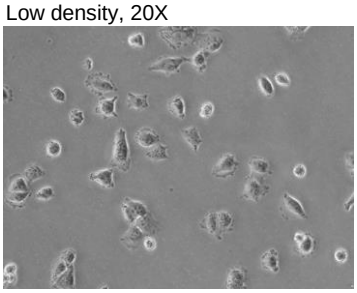
Certificate of Analysis

Testing performed by Imanis Life Sciences

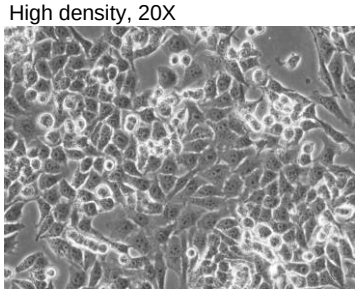
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
^{125}I uptake	Pass QC

Morphology:

Low density, 20X

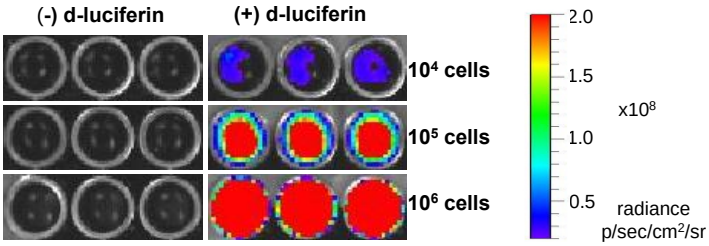


High density, 20X



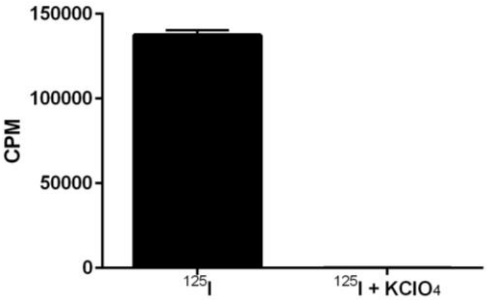
Low and high density photos taken 25 and 70 hours after thawing, respectively.

Luciferase Expression:



10^4, 10^5, or 10^6 cells were placed in wells of a 96-well plate and 0.3 mg of d-luciferin was added to the indicated wells. The plate was immediately imaged using a Xenogen IVIS Spectrum.

^{125}I Uptake:



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.

Legal Disclaimers

Limited Product Warranty
This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Imanis. Imanis shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

For *in vitro* use only. This certificate is a declaration of analysis at the time of manufacture.

PURCHASER NOTIFICATION

LIMITED LICENSE NOTICE – RESEARCH USE ONLY

IMANIS LIFE SCIENCES HAS A LIMTED LICENSE UNDER PATENTS OWNED BY THE SALK INSTITUTE FOR BIOLOGICAL STUDIES THAT PERMITS IMANIS LIFE SCIENCES TO SELL PRODUCTS CONTAINING WPRE FOR RESEARCH USE ONLY AND NOT FOR ANY COMMERCIAL USES. EXCLUDED COMMERCIAL USES INCLUDE WITHOUT LIMITATION MANUFACTURING, PROVIDING A SERVICE, THERAPEUTIC, DIAGNOSTIC AND PROPHYLACTIC USES, AND ANY OTHER COMMERCIAL USES. USE OF THIS PRODUCT BY A PURCHASER FOR ANY PURPOSE OTHER THAN FOR RESEARCH IS UNAUTHORIZED AND PROHIBITED.

The Salk Institute actively licenses its patents for commercial uses, and a commercial use license may be available for Salk's WPRE patents. If you wish to inquire about such a license, please contact:

Office of Technology Development
The Salk Institute for Biological Studies
10010 North Torrey Pines Road
La Jolla, CA 92037
Phone: (858) 453-4100 extension 1278
Fax: (858) 546-8093

THE NIS GENE AND TECHNOLOGY IS COVERED UNDER AN EXCLUSIVE LICENSE TO IMANIS LIFE SCIENCES. RESEARCHERS MAY USE THIS PRODUCT FOR RESEARCH USE ONLY AND NO COMMERCIAL USE IS ALLOWED. NO OTHER USE OR TRANSFER OF THIS PRODUCT OR DERIVATIVES IS AUTHORIZED WITHOUT THE PRIOR EXPRESS WRITTEN CONSENT OF IMANIS. WITH RESPECT TO ANY USES OUTSIDE THIS LABEL LICENSE, INCLUDING ANY DIAGNOSTIC, THERAPEUTIC OR PROPHYLACTIC USES, PLEASE CONTACT IMANIS LIFE SCIENCES FOR SUPPLY AND LICENSING INFORMATION.

Chief Operations Officer
Imanis Life Sciences
221 1st Ave SW STE 102
Rochester MN 55902
Tel: (507) 218-2559
Email: info@imanislife.com

Quality control by: RLV
Quality Assurance by: SPR
Effective Date: 12/15/15