B16F10-Fluc-hNIS

Product Description

Product Name: B16F10-Fluc-hNIS
Catalog Number: CL139
Lot Number: CL-IM153

Species: Mouse (*Mus musculus*)
Strain: C57BL/6
Cell type: Melanoma
Parental cells: B16F10 (ATCC® CRL-6475™)*
Morphology: Epithelial
Growth mode: Adherent
Reporter genes: Firefly luciferase (Fluc)
Human sodium iodide symporter (hNIS)

This is a population derived from the melanoma B16F10 cell line (ATCC® CRL-6475™). Parental B16F10 cells were transduced with LV-Luc2-P2A-hNIS (Imanis #LV023) encoding the firefly luciferase (Luc2; 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. High Fluc and hNIS expressing cells were selected 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 B16F10 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. The hNIS transgene facilitates high resolution, 3D SPECT/PET imaging of implanted cells. Fluc is 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 -130ºC).

Complete Growth Medium

Dulbecco's Modified Eagle's Medium (DMEM)
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 ~250 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium. Transfer cells to a T75 flask containing 10 mL complete growth medium.
5. Incubate the culture at 37°C with 5% CO_2. After 48 hours, replace the culture supernatant with fresh complete growth medium. Cells should reach full confluency 3-4 days after thawing.

Subculturing Instructions

Volumes are given for a T75 flask; increase or decrease as needed.
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 37ºC until cells have dissociated (approx. 2-3 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 2-4 days.

Freezing Medium

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

Additional Considerations

B16F10 cells produce melanin; accumulation of melanin turns the cells and culture medium dark brown or black. Melanin is toxic and B16F10 cells will die in the presence of excess melanin. Culture medium should be changed as soon as it becomes black, even if the cells are not confluent. Typically, media changes between passages are not required.

B16F10-Fluc-hNIS

Certificate of Analysis

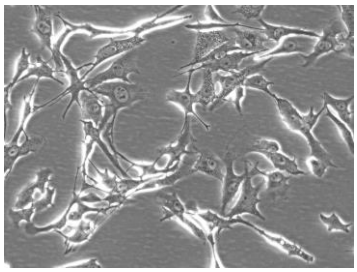
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	98%
Cells per vial	~ 5.5 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Luciferase expression	Pass QC
NIS expression	Pass QC
Average Doubling Time	21.1 hours*

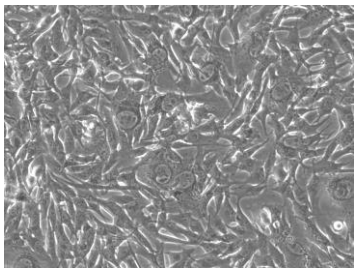
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology

Low density, 200X

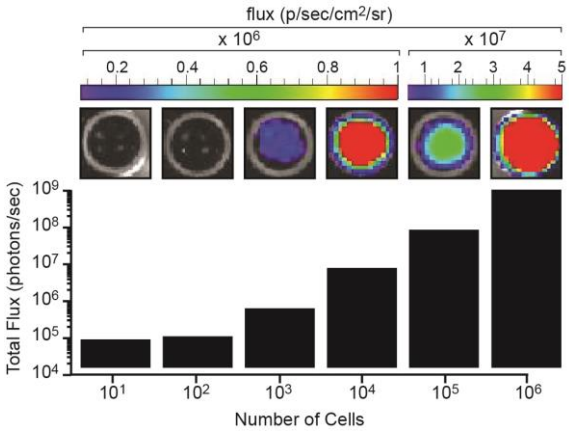


High density, 200X



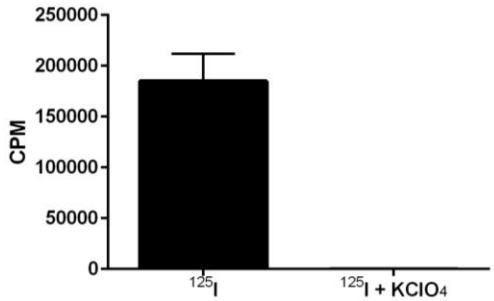
Low and high density photos taken at various times after thawing.

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.

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

THE IMANIS CELL LINES ARE NOT INTENDED FOR USE IN HUMANS. CELL LINES TRANSDUCED WITH LENTIVIRAL VECTORS ARE CLASSIFIED AS BIOSAFETY LEVEL 2 REAGENTS AND SHOULD BE USED UNDER THE APPROPRIATE BIOSAFETY LEVEL PER INSTITUTIONAL GUIDELINES.

THE PURCHASER AGREES THAT IMANIS MATERIALS DESIGNATED AS BIO-SAFETY LEVEL 2 CONSTITUTE KNOWN PATHOGENS AND THAT OTHER IMANIS MATERIALS NOT SO DESIGNATED AND ANY PROGENY OR MODIFICATION MAY BE PATHOGENIC UNDER CERTAIN CONDITIONS. PURCHASER ASSUMES ALL RISK AND RESPONSIBILITY IN CONNECTION WITH THE RECEIPT, HANDLING, STORAGE, DISPOSAL, TRANSFER AND USE OF THE IMANIS MATERIALS INCLUDING WITHOUT LIMITATION TAKING ALL APPROPRIATE SAFETY AND HANDLING PRECAUTIONS TO MINIMIZE HEALTH OR ENVIRONMENTAL RISK. PURCHASER AGREES THAT ANY ACTIVITY UNDERTAKEN WITH THE IMANIS MATERIALS AND ANY PROGENY OR MODIFICATION WILL BE CONDUCTED IN COMPLIANCE WITH ALL APPLICABLE GUIDELINES, LAWS AND REGULATIONS.

THE IMANIS MATERIAL, ANY OTHER IMANIS PRODUCTS, AND ANY TECHNICAL INFORMATION AND ASSISTANCE PROVIDED BY IMANIS ARE PROVIDED "AS IS", WITHOUT WARRANTIES OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, MANUFACTURE ACCORDING TO cGMP STANDARDS, TYPICALITY, SAFETY, ACCURACY AND NON-INFRINGEMENT.

IN NO EVENT SHALL IMANIS, ITS PARENTS, SUBSIDIARIES, DIRECTORS, OFFICERS, AGENTS, EMPLOYEES, ASSIGNS, SUCCESSORS AND AFFILIATE (COLLECTIVELY "IMANIS INDEMNIFIED PARTIES") BE LIABLE FOR INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES OF ANY KIND IN CONNECTION WITH OR ARISING OUT OF THIS AGREEMENT (WHETHER IN CONTRACT, TORT, NEGLIGENCE, STRICT LIABILITY, STATUTE OR OTHERWISE) EVEN IF IMANIS HAS BEEN ADVISED, KNEW OR SHOULD HAVE KNOWN OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING, BUT NOT LIMITED TO, LOST PROFITS, COST OF CAPITAL, COST OF SUBSTITUTE PRODUCTS OR CLAIMS OF LICENSEE'S CUSTOMERS FOR SUCH DAMAGE. IN NO EVENT SHALL IMANIS' CUMULATIVE LIABILITY EXCEED THE ACTUAL AMOUNTS PAID BY PURCHASER UNDER THIS AGREEMENT FOR THE TWELVE (12) MONTH PERIOD PRECEDING THE DATE OF THE EVENT GIVING RISE TO THE CLAIM. THE PROVISIONS OF THIS SECTION SHALL SURVIVE THE EXPIRATION OR TERMINATION OF THIS AGREEMENT AND SHALL APPLY EVEN IF THE LIMITED REMEDY SPECIFIED IN THIS AGREEMENT IS FOUND TO HAVE FAILED OF ITS ESSENTIAL PURPOSE.

Quality control by: CDL
Quality Assurance by: RLV
Effective Date: 26-Oct-2022