B16F10-Fluc-Neo/iRFP-Puro

Product Description

Product Name: B16F10-Fluc-Neo/iRFP-Puro
Catalog Number: CL110
Lot Number: CL-IM112

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)
Near infrared fluorescent protein (iRFP)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

This is a polyclonal population derived from the melanoma B16F10 cell line (ATCC® CRL-6475™). Parental B16F10 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-iRFP-P2A-Puro (Imanis #LV032) encoding the near-infrared fluorescent protein (iRFP; ex/em = 690/713) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High Fluc and iRFP expressing cells were selected using 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].

* The ATCC trademark and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection

Mycoplasma Testing

The B16F10-Fluc-Neo/iRFP-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 B16F10 cell line used to generate B16F10-Fluc-Neo/iRFP-Puro, was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

Recommended Uses

B16F10-Fluc-Neo/iRFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation. B16F10 cells form tumors and pulmonary metastases post implantation into C57BL/6 mice[6]. The Fluc and iRFP transgenes facilitate noninvasive imaging 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]Fidler. Cancer Res, 1975. 35:218-224.

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
0.8 mg/mL G418 (to maintain high Fluc expression)
1 µg/mL Puromycin (to maintain high iRFP expression)

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 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 0.8 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. In order to maintain high Fluc and iRFP expression, it is recommended that cells be subcultured in the presence of 0.8 mg/mL G418 and 1 µg/mL puromycin.

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. 2-5 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. If the growth medium begins to turn brown before cells are ready to be passaged, replace with fresh medium.

Freezing Medium

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.

Additional Considerations

Over trypsinization of B16F10 cells can damage the cells. During trypsinization the cells should be monitored carefully, and the trypsin neutralized immediately upon cell detachment.

B16F10-Fluc-Neo/iRFP-Puro

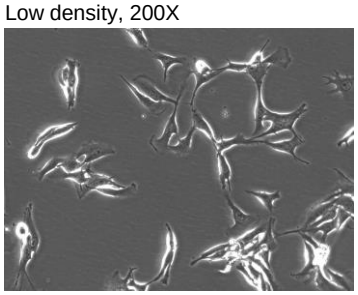
Certificate of Analysis

Testing performed by Imanis Life Sciences

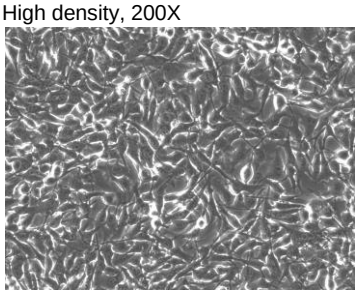
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC

Morphology:

Low density, 200X

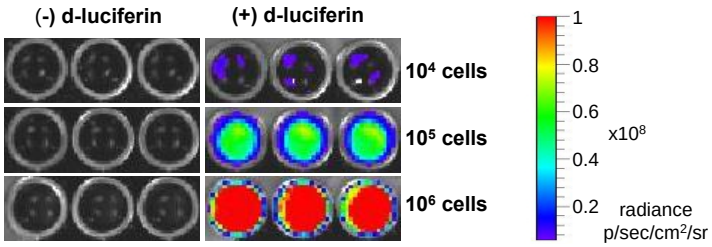


High density, 200X



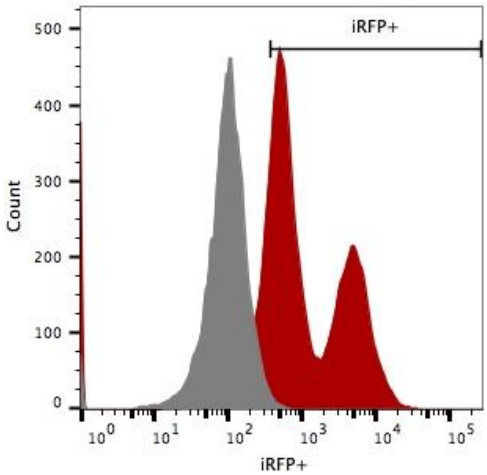
Low and high density photos taken 24 and 48 hours after thawing, respectively.

Luciferase Expression



10^4, 10^5, or 10^6 cells were placed in wells of a 96-well plate and 3 mg/mL of d-luciferin was added to the indicated wells. The plate was immediately imaged using a Xenogen IVIS Spectrum.

Fluorescence Expression



B16F10-Fluc-Neo/iRFP-Puro (red) or isotype control (B16F10; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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 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: RLV
Quality Assurance by: SPR
Effective Date: Feb-11-2016