B16F10-Fluc-Neo/iRFP-Puro

Product Description

Product Name: B16F10-Fluc-Neo/iRFP-Puro
Catalog Number: CL110
Lot Number: CL-IM184

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 and G418. 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].

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Mycoplasma Testing

This cell line has tested negative for mycoplasma contamination.

Cell Line Authentication

Authentication of the parental B16F10 cell line was confirmed by short tandem repeat (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 iRFP transgene facilitates *in vivo* noninvasive fluorescence imaging of implanted cells.

Fluc and iRFP are 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
0.8 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 (~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. 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 pre-warmed complete growth medium.
5. Incubate the culture at 37°C with 5% CO_2. Cells should reach full confluency 1-2 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 room temperature 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 1:12 is recommended. At this ratio cells will be ready for passage approximately every 3 days.

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 G418 and puromycin 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.

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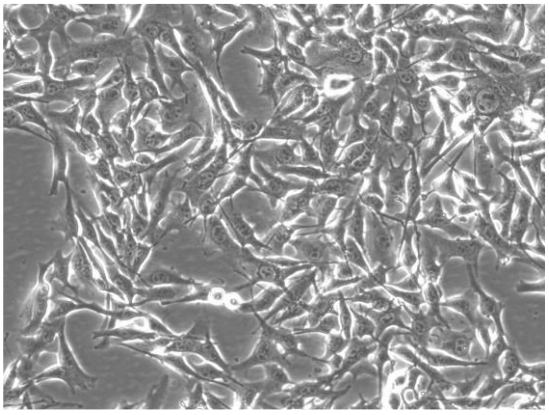
Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	99%
Sterility	No contamination detected
Mycoplasma	Negative
G418 selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
iRFP expression	Pass QC
Average doubling time	13.9 h*

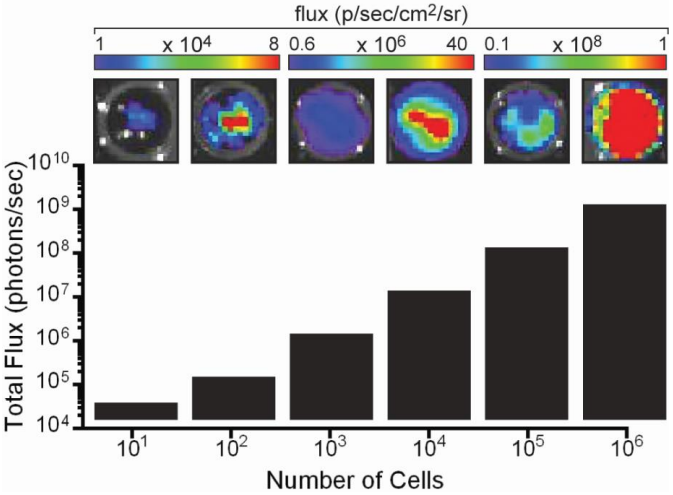
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:



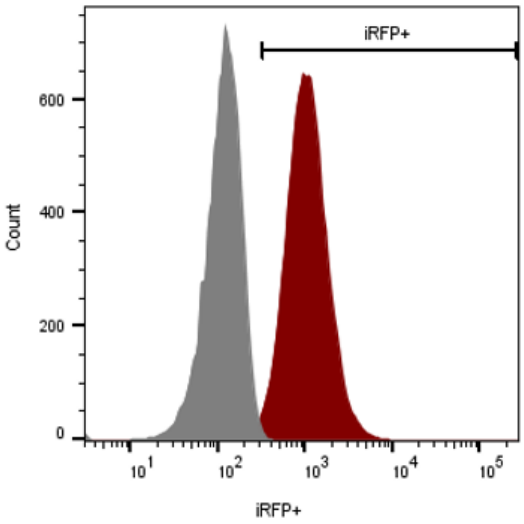
200X photo taken after thawing.

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.

Fluorescence Expression



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

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