B16F10-iRFP-Puro

Product Description

Product Name: B16F10-iRFP-Puro
Catalog Number: CL099
Lot Number: CL-IM97

Species: Mouse (*Mus musculus*)
Strain: C57BL/6
Cell type: Melanoma
Parental cells: B16F10 (ATCC® CRL-6475™)*
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Near infrared fluorescent protein (iRFP)
Selection gene: Puromycin (Puro)

This is a polyclonal population derived from the melanoma B16F10 cell line (ATCC® CRL-6475™). Parental B16F10 cells were transduced with 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 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].

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Mycoplasma Testing

The B16F10-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-iRFP-Puro, was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

Recommended Uses

B16F10-iRFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation.

B16F10 cells form tumors and pulmonary metastases post implantation into syngenic C57BL/6 mice[6]. The iRFP transgene facilitates 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
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 puromycin. Centrifuge cells at ~250 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium without puromycin. Transfer cells to a T75 flask containing 10 mL pre-warmed complete growth medium without puromycin.
5. Incubate the culture at 37°C with 5% CO_2. After 48 hours, replace the culture supernatant with complete growth medium containing 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 iRFP expression, it is recommended that cells be subcultured in the presence of 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 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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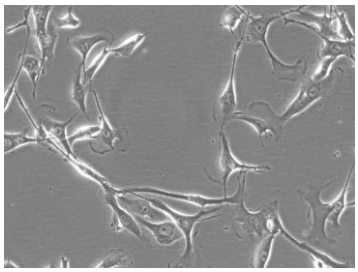
Certificate of Analysis

Testing performed by Imanis Life Sciences

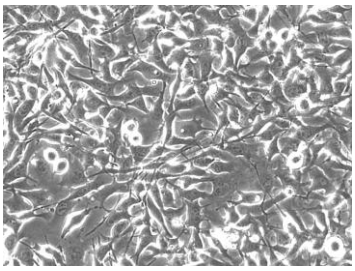
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC

Morphology:

Low density, 200X

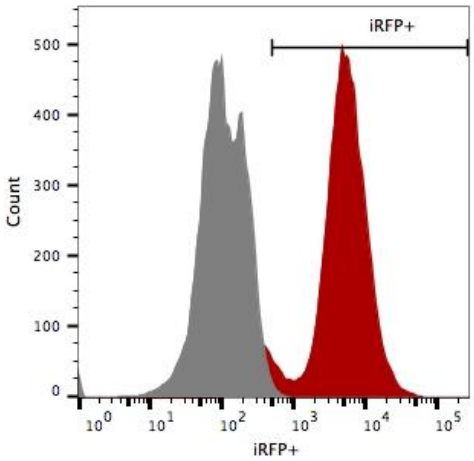


High density, 200X



Low and high density photos taken 24 and 72 hours after thawing, respectively.

Fluorescence Expression



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

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