

## Product Description

Product Name: B16F10-iRFP-Puro  
Catalog Number: CL099  
Lot Number: IMP015

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 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 (Miyoshi et al. J Virol. 1998. 72:8150-8157).

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## Mycoplasma Testing

This cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

## Cell Line Authentication

Authentication of the parental B16F10 cell line was confirmed by STR profiling.

## Recommended Uses

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

The iRFP transgene facilitates noninvasive imaging of implanted cells.

## Storage Instructions

Remove cells from the dry ice packaging and immediately store in the vapor phase above liquid nitrogen (below -130°C).

## Biosafety Notice

This cell line was generated by transduction with a lentiviral vector. Cell lines transduced with lentiviral vectors are classified as biosafety level 2 reagents and should be used under appropriate biosafety level for institutional guidelines.

## 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)

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 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 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 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<sub>2</sub>. 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 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<sub>2</sub> 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

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

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.

## Certificate of Analysis

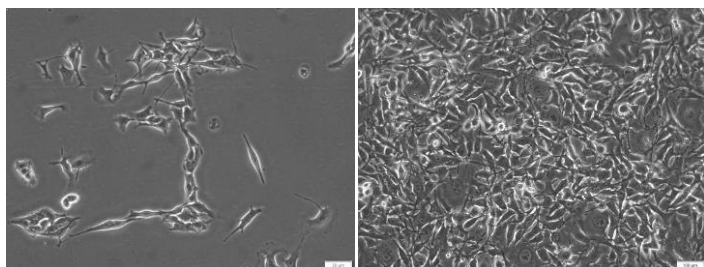
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	92%
Cells per vial	~ 1.1 x 10 <sup>7</sup>
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC

## Morphology

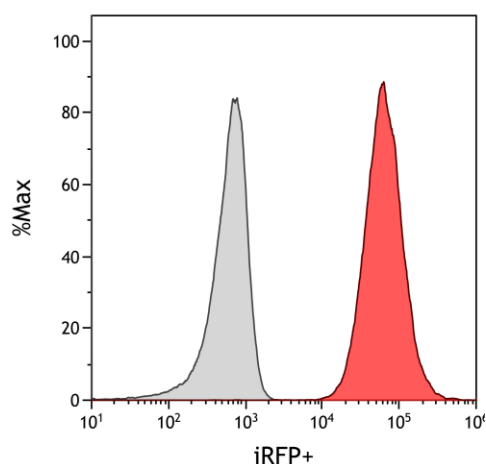
Low density, 200X

High density, 200X



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

## 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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Quality control by: AWD

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Effective Date: 27-Dec-2022