A549-iRFP-Puro

Product Description

Product Name: A549-iRFP-Puro
Catalog Number: CL082
Lot Number: CL-IM219

Species: Human (*Homo sapiens*)
Tissue: Lung
Cell type: Adenocarcinoma
Parental cells: A549 (ATCC® CCL-185™)
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Near infrared fluorescent protein (iRFP)
Selection gene: Puromycin (Puro)

This is a polyclonal population derived from the adenocarcinoma A549 cell line (ATCC® CCL-185™). Parental A549 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].

Mycoplasma Testing

The A549-iRFP-Puro cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental A549 cell line used to generate A549-iRFP-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

Recommended Uses

A549-iRFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation.

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]Jiang et al. Oncogene. 2001. 20:2254-2263.
[7]Jenkins et al. Clin & Exp Metastasis. 2003. 20:733-744.

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)

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 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 2-3 days after thawing.

Subculturing Instructions

Volumes are given for a T75 flask; increase or decrease as needed. To maintain high iRFP expression, it is recommended that cells be subcultured in the presence of 1 µg/mL puromycin. A549-iRFP-Puro cells should be passaged when they reach 90-100% confluency.

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 1:8 is recommended. At this ratio cells will be ready for passage every 3-4 days.

Freezing Medium

A549-iRFP-Puro 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.

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Certificate of Analysis

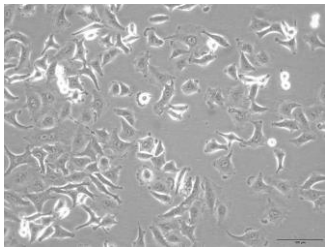
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	99%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC
Average Doubling Time	20.3 hours*

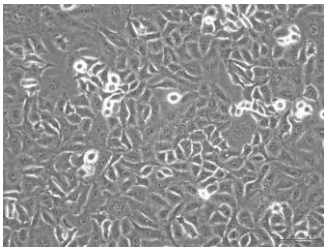
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:

Low density, 20X

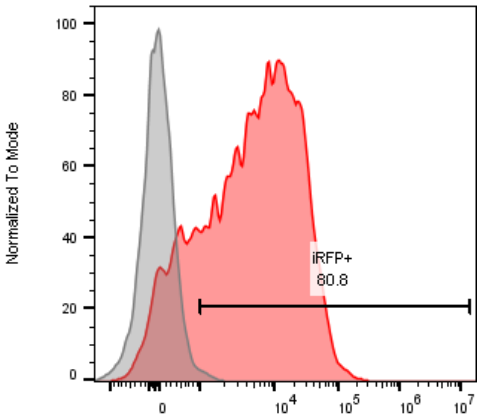


High density, 20X



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

Fluorescence Expression



A549-iRFP-Puro (red) or isotype control (A549-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

Quality control by: CMD
Quality Assurance by: RLV
Effective Date: 07-Jan-2022

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