

# A549-Fluc-Neo/iRFP-Puro

## Product Description

Product Name: A549-Fluc-Neo/iRFP-Puro  
Catalog Number: CL098  
Lot Number: IMP026

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

This is a cell line derived from the A549 cell line (ATCC® CCL-185™). Parental A549 cells were transduced with, 1) LV-SFFV-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 SFFV promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High Fluc and iRFP expressing cells were selected using G418 and 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<sup>1</sup>.

## Mycoplasma Testing

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

## Cell Line Authentication

Authentication of the parental A549 cell line was confirmed by short tandem repeat (STR) profiling.

## Recommended Uses

These cells are suitable for *in vitro* and *in vivo* experimentation.

## References

<sup>1</sup>Miyoshi et al. J Virol. 1998. 72:8150-8157.

## Biosafety Notice

These cell lines were 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.

## Storage Instructions

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

## Complete Growth Medium

Dulbecco's Modified Eagle's Medium (DMEM)  
10% Fetal Bovine Serum (FBS)  
1% Penicillin/Streptomycin  
0.6 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 37°C water bath. To limit contamination, do not submerge the O-ring and cap.
2. When cells are ~70% thawed (about 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 4-5 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 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 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 1:8 is recommended. At this ratio cells will be ready for passage every 2-3 days.

## Freezing Medium

These cells can be amplified and used to generate additional frozen stocks. Cryopreservation of low passage stocks is recommended. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium without G418 and puromycin supplemented with 5-10% DMSO.

## Certificate of Analysis

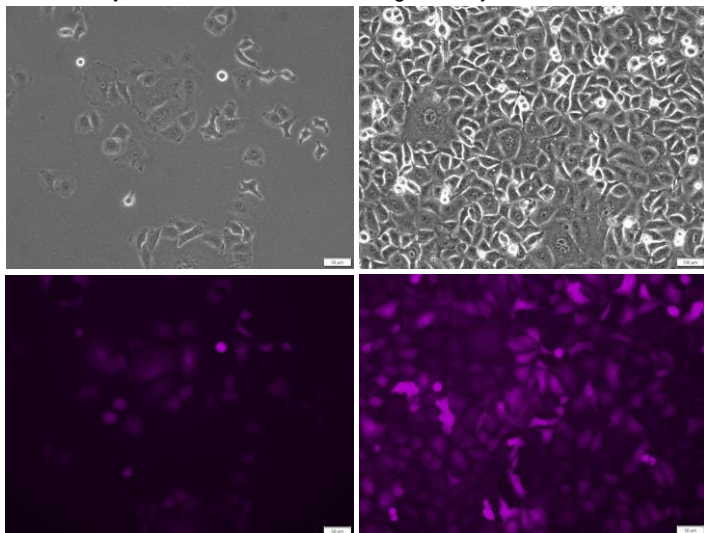
Testing performed by Imanis Life Sciences

| Test description               | Result                    |
|--------------------------------|---------------------------|
| Post thaw viable cell recovery | 98%                       |
| Viable cells per vial          | ~ 4.6 x 10 <sup>6</sup>   |
| Sterility                      | No contamination detected |
| Mycoplasma                     | No contamination detected |
| Luciferase expression          | Pass QC                   |
| Fluorescence expression        | Pass QC                   |

## Morphology:

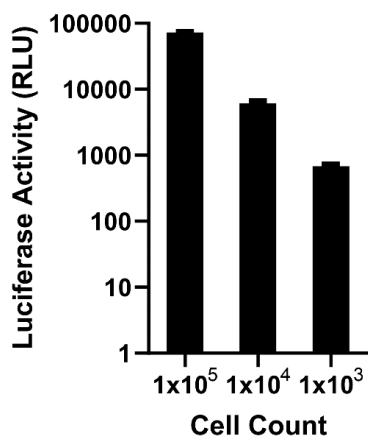
Low density, 200X

High density, 200X



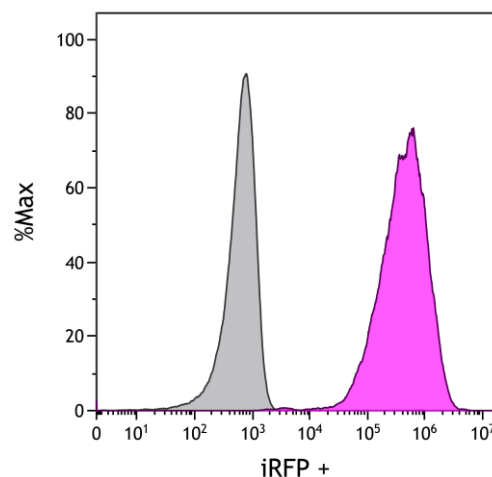
Low- and high-density photos taken at various times after thawing.

## Luciferase Expression



The indicated number of cells were placed in wells of a 96-well plate. After the addition of 15 mg/mL d-luciferin, bioluminescence was immediately imaged using a microplate reader.

## Fluorescence Expression



A549-Fluc-Neo/iRFP-Puro (purple) or parental control (grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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Quality control by: TFC

Quality Assurance by: RLV

Effective Date: 03-Oct-2023