

Product Description

Product Name: A549-Fluc-Neo/eGFP-Puro
Catalog Number: CL097
Lot Number: CL-IM95

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)
Enhanced green fluorescent protein (eGFP)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

This is a polyclonal population derived from the adenocarcinoma A549 cell line (ATCC® CCL-185™). Parental A549 cells were transduced with 1) LV-Fluc-P2A-Puro (Imanis #LV011) encoding the firefly luciferase (Fluc) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene (Puro) via a P2A cleavage peptide and 2) LV-eGFP-PGK-Puro (Imanis #LV031) encoding the enhanced green fluorescent protein (eGFP) cDNA under the SFFV promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter. High Fluc and eGFP 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 LTR¹.

Mycoplasma Testing

The A549-Fluc-Neo/eGFP-Puro cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental A549 cell line was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

Recommended Uses

A549-Fluc-Neo/eGFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation.

The Fluc and eGFP transgenes in the A549-Fluc-Neo/eGFP-Puro cells facilitate *in vivo* noninvasive imaging and optical imaging, respectively, of implanted cells. In-life imaging for GFP fluorescence is not recommended due to high background autofluorescence. Tissues may be harvested post mortem for analysis by conventional microscopy.

References

¹Miyoshi 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 -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 (to maintain high Fluc expression)
1 µg/mL puromycin (to maintain high eGFP expression)

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 (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 selection drugs. Centrifuge cells at ~250 x g for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium without selection drugs. Transfer cells to a T75 flask containing 10 mL pre-warmed complete growth medium without selection drugs.
5. Incubate the culture at 37°C with 5% CO₂. After 48 hours, replace the culture supernatant with complete growth medium containing 0.6 mg/mL G418 and 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. To maintain high Fluc and eGFP expression, it is recommended that cells be subcultured in the presence of 0.6 mg/mL G418 and 1 µg/mL puromycin. A549-Fluc-Neo/eGFP-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 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% CO₂ 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-Fluc-Neo/eGFP-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 selection drugs supplemented with 5-10% DMSO.

Certificate of Analysis

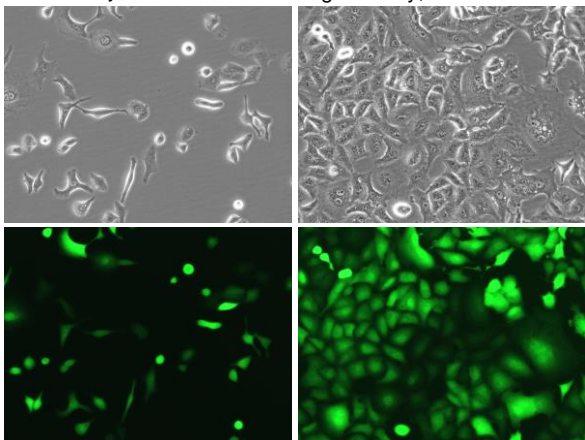
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viability	98%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC

Morphology:

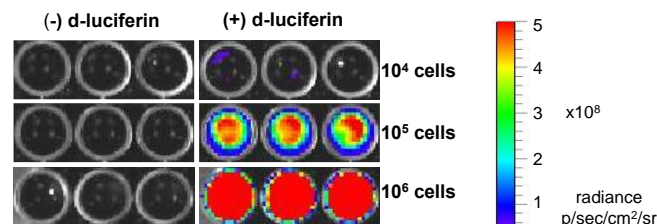
Low density, 20X

High density, 20X



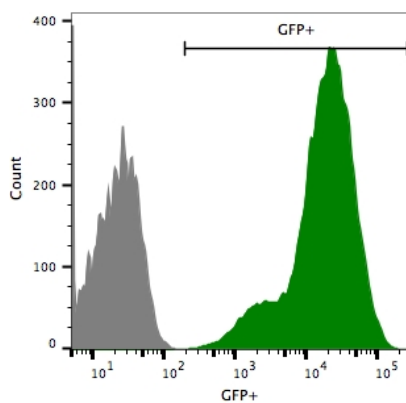
Low and high density photos taken 24 and 72 hours after thawing, respectively.

Luciferase Expression



10^4 , 10^5 , or 10^6 cells were placed in wells of a 96-well plate and 0.3 mg of d-luciferin was added to the indicated wells. The plate was immediately imaged using a Xenogen IVIS Spectrum.

Fluorescence Expression



A549-Fluc-Neo/eGFP-Puro (green) or isotype control (A549-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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Quality control by: AMW

Quality Assurance by: RLC

Effective Date: 26-Oct-2022