

# A375-Fluc-Neo/eGFP-Puro

## Product Description

Product Name: A375-Fluc-Neo/eGFP-Puro  
 Catalog Number: CL085  
 Lot Number: CL-IM82

Species: Human (*Homo sapiens*)  
 Tissue: Skin  
 Cell type: Malignant melanoma  
 Parental cells: A375 (ATCC® CRL-1619™)  
 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 malignant melanoma A375 cell line (ATCC® CRL-1619™). Parental A375 cells were transduced with 1) LV-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 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 LTR<sup>1</sup>.

## Mycoplasma Testing

The A375-Fluc-Neo/eGFP-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,<sup>2,3</sup> several funding organizations, including NIH, as well as major publishers, including those affiliated with the American Association for Cancer Research (AACR), require cell lines used in research to be authenticated prior to publication<sup>4,5</sup>. The parental A375 cell line used to generate A375-Fluc-Neo/eGFP-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

## Recommended Uses

A375-Fluc-Neo/eGFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation. A375 cells form tumors post implantation into immunosuppressed mice<sup>6</sup>. The Fluc transgene in the A375-Fluc-Neo/eGFP-Puro cells facilitates noninvasive bioluminescent imaging of implanted cells, while the eGFP gene facilitates conventional microscopy of excised tissue.

## References

- <sup>1</sup>Miyoshi et al. J Virol. 1998. 72:8150-8157.
- <sup>2</sup>Hughes et al. BioTechniques 2007. 43: 575-586.
- <sup>3</sup>Chatterjee et al. Science 2007. 315:928-931.
- <sup>4</sup><https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html>
- <sup>5</sup><http://www.aacrjournals.org/site/InstraAuthors/fora.xhtml#celllineuse>
- <sup>6</sup>Gershwin et al. J Natl Cancer Inst. 1977. 58:1455-1461.

## 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)

## 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% CO<sub>2</sub>. 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. A375-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% 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

A375-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.

# A375-Fluc-Neo/eGFP-Puro

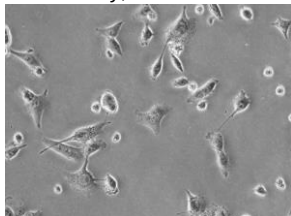
## Certificate of Analysis

Testing performed by Imanis Life Sciences

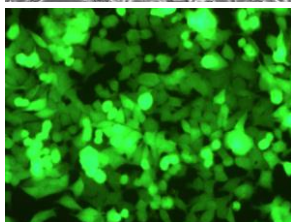
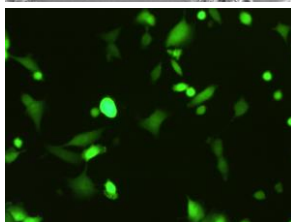
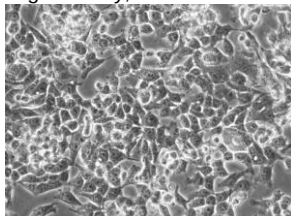
| Test description               | Result                    |
|--------------------------------|---------------------------|
| Post thaw viable cell recovery | Pass QC                   |
| Sterility                      | No contamination detected |
| Mycoplasma                     | No contamination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

## Morphology:

Low density, 20X

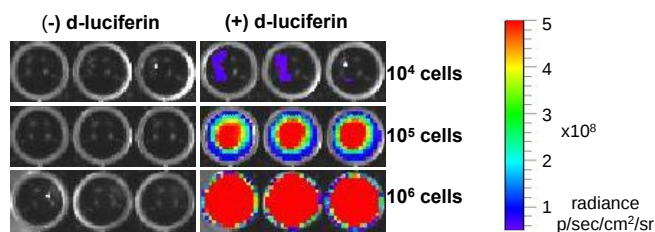


High density, 20X



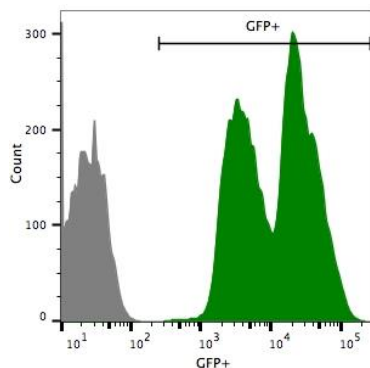
Low and high density photos taken 25 and 70 hours after thawing, respectively.

## Luciferase Expression:



10<sup>4</sup>, 10<sup>5</sup>, or 10<sup>6</sup> 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:



A375-Fluc-Neo/eGFP-Puro (green) or isotype control (A375-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry (20,000 events).

## Legal Disclaimers

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