

## Product Description

Product Name: A549-hNIS-Neo/Fluc-Puro  
Catalog Number: CL083  
Lot Number: CL-IM80

Species: Human (*Homo sapiens*)  
Tissue: Lung  
Cell type: Adenocarcinoma  
Parental cells: A549 (ATCC® CCL-185™)  
Morphology: Epithelial  
Growth mode: Adherent  
Reporter genes: Human sodium iodide symporter (hNIS)  
Firefly luciferase (Fluc)  
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-hNIS-IRES-Neo (Imanis #LV013) encoding the human sodium iodide symporter (hNIS) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene (Neo) via an IRES and 2) LV-Fluc-P2A-Puro (Imanis #LV012) encoding the firefly luciferase (Fluc) cDNA under the SFFV promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High hNIS and Fluc 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 tested negative for mycoplasma contamination.

## Cell Line Authentication

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

## Recommended Uses

This cell line is suitable for in vitro and in vivo experimentation.

## References

<sup>1</sup>Miyoshi 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 hNIS expression)  
1 µg/mL puromycin (to maintain high Fluc 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 (~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 without G418 or puromycin. 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 without G418 or puromycin.
5. Incubate the culture at 37°C with 5% CO<sub>2</sub>. Cells should reach full confluency approximately 3 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 room temperature 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 approximately every 3-4 days.

## Freezing Medium

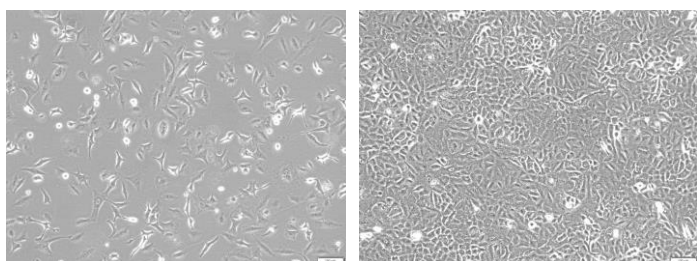
Cells can be amplified and used to generate additional frozen stocks. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium without G418 and puromycin supplemented with 5-10% DMSO.

## Certificate of Analysis

Testing performed by Imanis Life Sciences

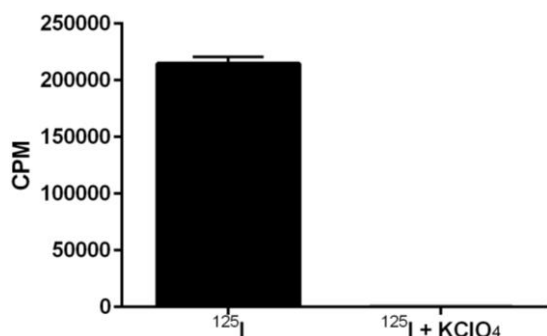
Test description	Result
Post thaw viable cell recovery	~ 2 x10 <sup>6</sup>
Post thaw cell viability	97%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
<sup>125</sup> I Uptake	Pass QC
Luciferase expression	Pass QC

## Morphology:



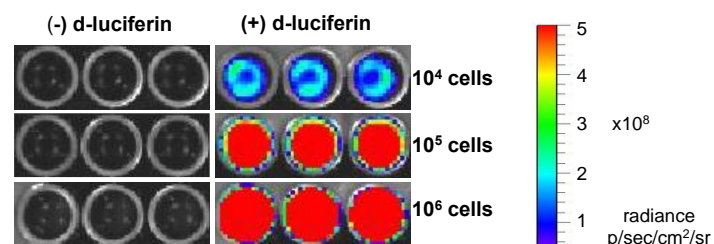
Cells photos taken at 100x magnification at 24 hours (left) and 72 hours (right) post thaw.

## <sup>125</sup>I Uptake



Uptake of <sup>125</sup>I by 3 x 10<sup>5</sup> cells was assayed in the presence or absence of KClO<sub>4</sub>, an inhibitor of NIS-mediated <sup>125</sup>I uptake.

## 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.

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