A549-hNIS-Neo

Product Description

Product Name: A549-hNIS-Neo
Catalog Number: CL004
Lot Number: CL-MA04

Species: Human (*Homo sapiens*)
Strain: Lung
Cell type: Adenocarcinoma
Parental cells: A549 (ATCC® CCL-185™)*
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Human sodium iodide symporter (hNIS)
Selection gene: Neomycin (Neo)

A549-hNIS-Neo is a polyclonal population of the human lung carcinoma A549 cell line transduced with a lentiviral vector (Imanis #LV013) encoding the human sodium iodide symporter (hNIS) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene via an IRES. High NIS expressing cells were selected using G418. 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].

* The ATCC trademark and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection

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

In vitro: This is a high hNIS expressing clone suitable for use as a positive control cell line in I-125 uptake assays to validate NIS expression in your lentiviral transduced cells.

In vivo: A549 cells form metastases in the lungs of mice post systemic administration. The in vivo growth of these metastases can be monitored noninvasively in animals using SPECT or PET imaging

References

[1]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 -130ºC).

Complete Growth Medium

Dulbecco's Modified Eagle's Medium (DMEM)
10% Fetal bovine serum (FBS)
1% Penicillin/Streptomycin
0.6 mg/mL G418

G418 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 to the growth medium.

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 (~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. 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 pre-warmed complete growth medium.
5. Incubate the culture at 37°C with 5% CO_2. 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 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_2 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 supplemented with 5-10% DMSO.

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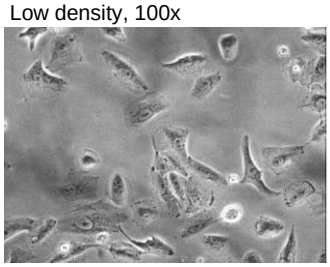
Certificate of Analysis

Testing performed by Imanis Life Sciences:

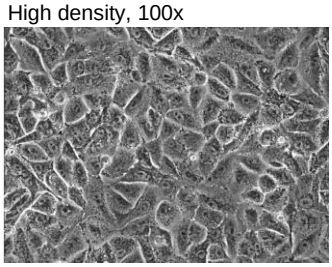
Test description	Result
Post thaw viable cell recovery	95%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
^{125}I uptake assay	Pass QC

Morphology:

Low density, 100x

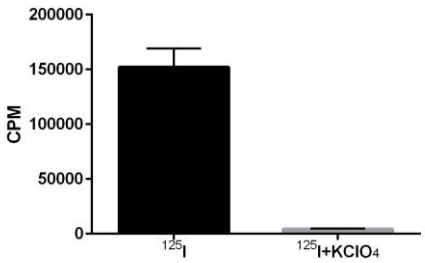


High density, 100x



Low and high density photos taken at various times.

^{125}I uptake:



Uptake of ^{125}I by 2 x 10^5 cells was assayed in the presence or absence of $KClO_4$, an inhibitor of NIS-mediated ^{125}I uptake.

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