

HCT116-hNIS-Neo/Fluc-Puro

Product Description

Product Name: HCT116-hNIS-Neo/Fluc-Puro
Catalog Number: CL009
Lot Number: CL-IM46

Species: Human (*Homo sapiens*)
Strain: Colorectal
Cell type: Colorectal carcinoma
Parental cells: HCT116 (ATCC® CCL-247™)*
Morphology: Epithelial
Growth mode: Adherent
Reporter genes: Human sodium iodide symporter (hNIS)
Firefly luciferase (Fluc)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

HCT116-hNIS-Neo/Fluc-Puro is a polyclonal population of the human colorectal carcinoma HCT116 cell line transduced with lentiviral vectors encoding 1) 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 (Imanis #LV013) and 2) the firefly luciferase (Fluc) cDNA under the SFFV promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide (Imanis #LV012). 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 LTR¹.

* 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 HCT116 cell line was confirmed by short tandem repeat (STR) profiling.

Recommended Uses

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

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
0.5 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 (~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% CO₂. 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% CO₂ 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.

Additional Considerations

Cells take longer to attach. Once attached, cells will still be rounded for over 24 hours and then start to flatten down more.

Certificate of Analysis

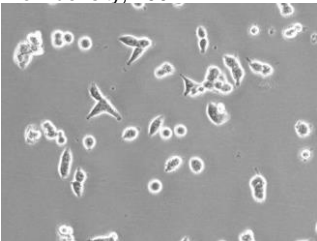
Testing performed by Imanis Life Sciences:

Test description	Result
Post thaw viable cell recovery	98%
Cells/vial	~6x10 ⁶
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
¹²⁵ I Uptake assay	Pass QC
Luciferase expression	Pass QC
Average doubling time*	14.2 hrs

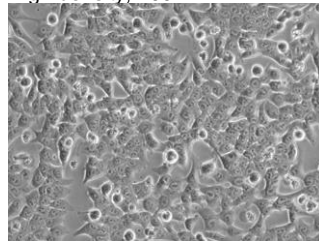
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:

Low density, 200X

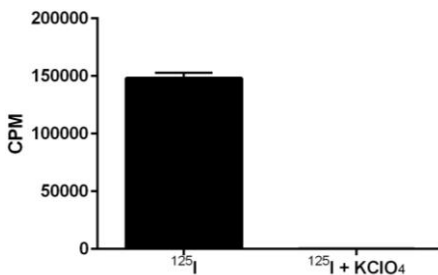


High density, 200X



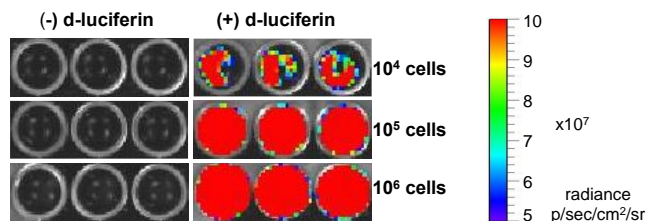
Low and high density photos taken at various times.

¹²⁵I uptake:



Uptake of ¹²⁵I by 3 x 10⁵ cells was assayed in the presence or absence of KClO₄, an inhibitor of NIS-mediated ¹²⁵I uptake.

Luciferase Expression:



The indicated number of cells were placed in wells of a 96-well plate. After the addition of 3 mg/mL d-luciferin, the plate was immediately imaged using a Xenogen IVIS Spectrum. The total flux (photons/sec) was plotted as a function of cell number.

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Quality control by: CDL

Quality Assurance by: RLV

Effective Date: 31-Aug-2021