HT1080-hNIS-Neo/Fluc-Puro

Product Description

Product Name: HT1080-hNIS-Neo/Fluc-Puro
Catalog Number: CL105
Lot Number: CL-IM103

Species: Human (*Homo sapiens*)
Tissue: Connective tissue
Cell type: Fibrosarcoma
Parental cells: HT1080 (ATCC® CCL-121™)
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 fibrosarcoma HT1080 cell line (ATCC® CCL-121™). Parental HT1080 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[1].

Mycoplasma Testing

The HT1080-hNIS-Neo/Fluc-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,[2,3] several funding organizations, including NIH, as well as major publishers, including those affiliated with the American Associate for Cancer Research (AACR), require cell lines used in research to be authenticated prior to publication[4,5]. The parental HT1080 cell line used to generate HT1080-hNIS-Neo/Fluc-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

Recommended Uses

HT1080-hNIS-Neo/Fluc-Puro cells are suitable for *in vitro* and *in vivo* experimentation. HT1080 cells form primary tumors and distant metastases (lung, liver, and brain) post implantation into immunosuppressed mice[6,7]. The hNIS and Fluc transgenes facilitate non-invasive, high-resolution 3D PET/SPECT and non-invasive bioluminescent imaging, respectively, of implanted cells.

References

[1]Miyoshi et al. J Virol. 1998. 72:8150-8157.
[2]Hughes et al. BioTechniques 2007. 43: 575-586.
[3]Chatterjee et al. Science 2007. 315:928-931.
[4]https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html
[5]http://www.aacrjournals.org/site/InstrAuthors/ifora.xhtml#celllineuse
[6]Rasheed et al. Cancer. 1974. 33:1027-1033.
[7]Praus et al. Gene Therapy. 1999. 6:227-236.

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
1.25 mg/mL G418 (to maintain high hNIS expression)
1 µg/mL puromycin (to maintain high Fluc 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_2. After 48 hours, replace the culture supernatant with complete growth medium containing 1.25 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 hNIS and Fluc expression, it is recommended that cells be subcultured in the presence of 1.25 mg/mL G418 and 1 µg/mL puromycin. HT1080-hNIS-Neo/Fluc-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_2 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

HT1080-hNIS-Neo/Fluc-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.

Additional Considerations

HT1080-hNIS-Neo/Fluc-Puro cells can detach from tissue culture surfaces upon repeated washing. Coating culture plates with poly-D-Lysine prior to use can increase cell adherence to the plates during experiments requiring repeated washes or media changes.

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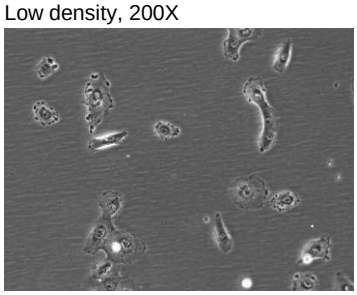
Certificate of Analysis

Testing performed by Imanis Life Sciences

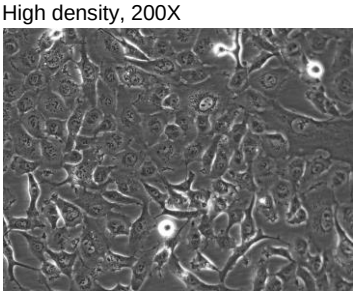
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
^{125}I uptake	Pass QC
Luciferase expression	Pass QC

Morphology:

Low density, 200X

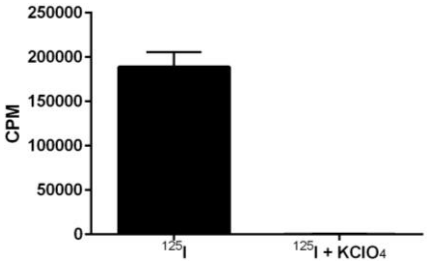


High density, 200X



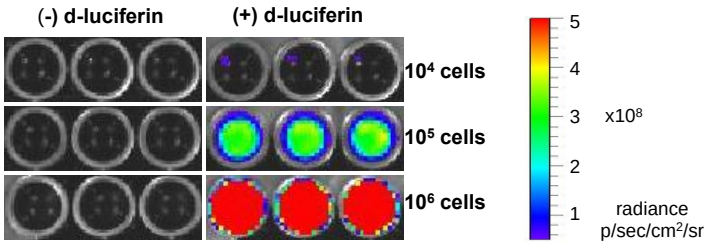
Low and high density photos taken 24 and 72 hours after thawing, respectively.

^{125}I Uptake



Uptake of ^{125}I by 3 x 10^5 cells was assayed in the presence or absence of $KClO_4$, an inhibitor of NIS-mediated ^{125}I uptake.

Luciferase Expression



10^4, 10^5, or 10^6 cells were placed in wells of a 96-well plate and 30 µg/mL of d-luciferin was added to the indicated wells. The plate was immediately imaged using a Xenogen IVIS Spectrum.

Quality control by: RLV
Quality Assurance by: SPR
Effective Date: 29-Jan-2016

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