HT1080-hNIS-Puro

Product Description

Product Name: HT1080-hNIS-Puro
Catalog Number: CL102
Lot Number: CL-IM100

Species: Human (*Homo sapiens*)
Tissue: Connective tissue
Cell type: Fibrosarcoma
Parental cells: HT1080 (ATCC® CCL-121™)
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Human sodium iodide symporter (hNIS)
Selection gene: Puromycin (Puro)

This is a polyclonal population derived from the fibrosarcoma HT1080 cell line (ATCC® CCL-121™). Parental HT1080 cells were transduced with LV-hNIS-P2A-Puro (Imanis #LV019) encoding the human sodium iodide symporter (hNIS) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High hNIS expressing cells were selected using 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

This cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental HT1080 cell line was authenticated and certified free of interspecies cross contamination by STR profiling.

Recommended Uses

HT1080-hNIS-Puro cells are suitable for *in vitro* and *in vivo* experimentation.

The hNIS transgene facilitates non-invasive, high-resolution 3D PET/SPECT imaging of implanted cells.

Storage Instructions

Remove cells from the dry ice packaging and immediately store in the vapor phase above liquid nitrogen (below -130ºC).

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
1 µg/mL puromycin (to maintain high hNIS expression)

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 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 37ºC 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 complete growth medium without puromycin. Centrifuge cells at ~250 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium without puromycin. Transfer cells to a T75 flask containing 10 mL complete growth medium without puromycin.
5. Incubate the culture at 37°C with 5% CO_2. After 48 hours, replace the culture supernatant with complete growth medium containing 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 expression, it is recommended that cells be subcultured in the presence of 1 µg/mL puromycin. Passage cells 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 37ºC 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-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 puromycin supplemented with 5-10% DMSO.

Additional Considerations

HT1080 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.

HT1080-hNIS-Puro

Certificate of Analysis

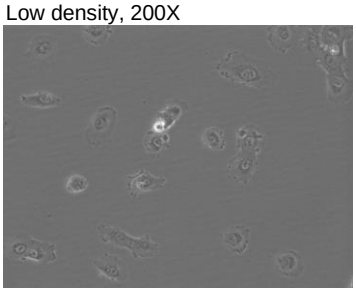
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	99%
Viable cells per vial	~ 3 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
^{125}I uptake	Pass QC
Average Doubling Time	22.1 hours*

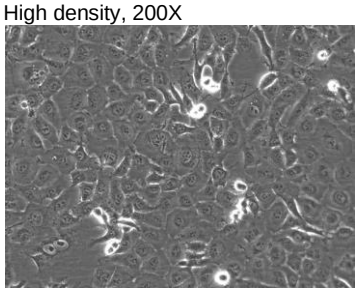
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:

Low density, 200X

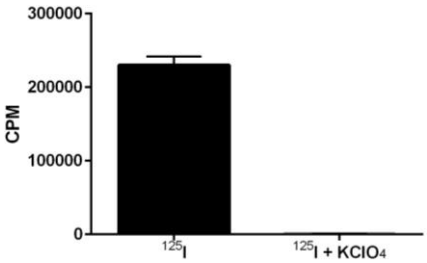


High density, 200X



Low- and high-density photos taken at various times after thawing.

^{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.

Legal Disclaimers

Limited Product Warranty
This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Imanis. Imanis shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

For *in vitro* use only. This certificate is a declaration of analysis at the time of manufacture.

PURCHASER NOTIFICATION

LIMITED LICENSE NOTICE – RESEARCH USE ONLY

THE NIS GENE AND TECHNOLOGY IS COVERED UNDER AN EXCLUSIVE LICENSE TO IMANIS LIFE SCIENCES. RESEARCHERS MAY USE THIS PRODUCT FOR RESEARCH USE ONLY AND NO COMMERCIAL USE IS ALLOWED. NO OTHER USE OR TRANSFER OF THIS PRODUCT OR DERIVATIVES IS AUTHORIZED WITHOUT THE PRIOR EXPRESS WRITTEN CONSENT OF IMANIS. WITH RESPECT TO ANY USES OUTSIDE THIS LABEL LICENSE, INCLUDING ANY DIAGNOSTIC, THERAPEUTIC OR PROPHYLACTIC USES, PLEASE CONTACT IMANIS LIFE SCIENCES FOR SUPPLY AND LICENSING INFORMATION.

Vice President of Operations
Imanis Life Sciences
2900 37th Street NW, Building 110
Rochester, MN 55901
Rochester MN 55902
Tel: (507) 218-2559
Email: support@imanislife.com

Quality control by: CDL
Quality Assurance by: RLV
Effective Date: 13-Oct-2022