HCT116-hNIS-Neo/eGFP-Puro

Product Description

Product Name: HCT116-hNIS-Neo/eGFP-Puro
Catalog Number: CL028
Lot Number: CL-IM51

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)
Enhanced green fluorescent protein (eGFP)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

HCT116-hNIS-Neo/eGFP-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 enhanced green fluorescent protein (eGFP) cDNA under the SFFV promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter (Imanis #LV031). High hNIS and eGFP-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].

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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.

The hNIS transgene facilitates *in vivo* noninvasive high-resolution 3D SPECT/PET imaging of implanted cells. eGFP is not recommended for whole animal in-live imaging. Rather, samples can be collected post mortem for analysis by conventional fluorescence microscopy.

eGFP is immunogenic and may cause tumor rejection in immunocompetent mice. For the most consistent results, immunocompromised mice are recommended for studies.

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
0.5 mg/mL G418
1 µg/mL Puromycin

Caution! Typical commercial puromycin stocks are provided at a concentration of 10 mg/mL or 10,000X.

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.

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.
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 2-3 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.

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Certificate of Analysis

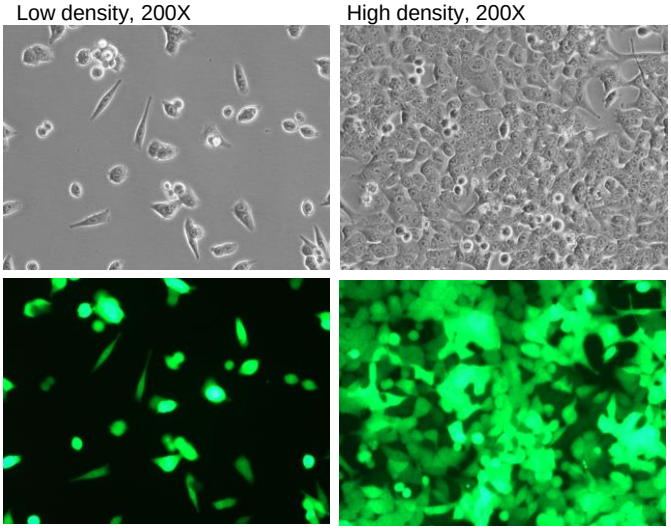
Testing performed by Imanis Life Sciences:

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 assay	Pass QC
Fluorescence expression	Pass QC

Morphology:

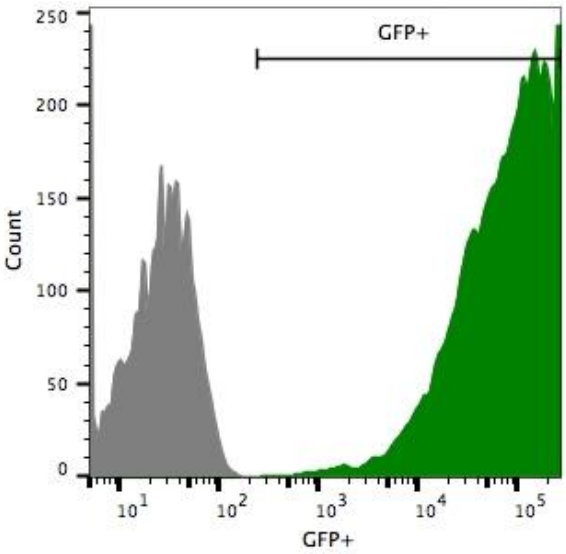
Low density, 200X

High density, 200X



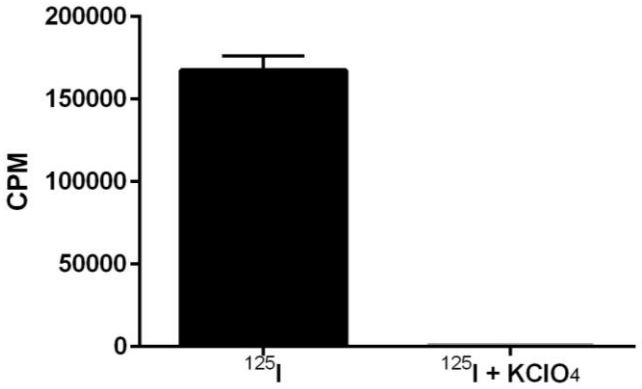
Low and high density photos taken at various times after thawing.

Fluorescence Expression:



HCT116-hNIS-Neo/eGFP-Puro (green) or isotype control (HCT116-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

^{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.

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