4T1-mNIS-Neo/iRFP-Puro

Product Description

Product Name: 4T1-mNIS-Neo/iRFP-Puro
Catalog Number: CL108
Lot Number: CL-IM106

Species: Mouse (*Mus musculus*)
Strain: BALB/cfC3H
Cell type: Mammary carcinoma
Parental cells: 4T1 (ATCC® CRL-2539™)
Morphology: Epithelial
Growth mode: Adherent
Reporter genes: Murine sodium iodide symporter (hNIS)
Near infrared fluorescent protein (iRFP)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

This is a polyclonal population derived from the mammary carcinoma 4T1 cell line (ATCC® CRL-2539™). Parental 4T1 cells were transduced with 1) LV-mNIS-P2A-Neo (Imanis #LV025) encoding the murine sodium iodide symporter (mNIS) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene (Neo) via a P2A cleavage peptide and 2) LV-iRFP-P2A-Puro (Imanis #LV032) encoding the near infrared fluorescent protein (iRFP; ex/em = 690/713) cDNA under the SFFV promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High mNIS and iRFP 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 4T1-mNIS-Neo/iRFP-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 4T1 cell line used to generate 4T1-mNIS-Neo/iRFP-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

Recommended Uses

4T1-mNIS-Neo/iRFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation.

4T1 cells form primary tumors that can metastasize to the lung, liver, lymph nodes, and brain post implantation into syngenic BALB/c mice[6]. The mNIS and iRFP transgenes in the 4T1-mNIS-Neo/iRFP-Puro cells facilitate *in vivo* high-resolution 3D PET/SPECT imaging and noninvasive fluorescent imaging, respectively, of implanted cells.

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

RPMI-1640 Medium (RPMI)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
0.1 mg/mL G418 (to maintain high mNIS expression)
2 µg/mL puromycin (to maintain high iRFP 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 0.1 mg/mL G418 and 2 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 mNIS and iRFP expression, it is recommended that cells be subcultured in the presence of 0.1 mg/mL G418 and 2 µg/mL puromycin. 4T1-mNIS-Neo/iRFP-Puro cells frequently clump and should be passaged when they reach 80-90% confluency overall.

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

4T1-mNIS-Neo/iRFP-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.

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]Pulaski and Ostrand-Rosenberg. Cancer Res. 1998. 58:1486-1493.

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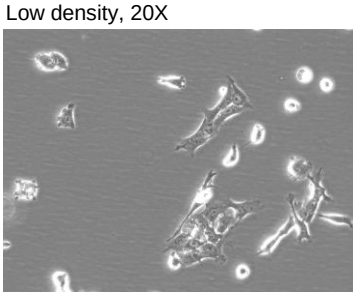
Certificate of Analysis

Testing performed by Imanis Life Sciences

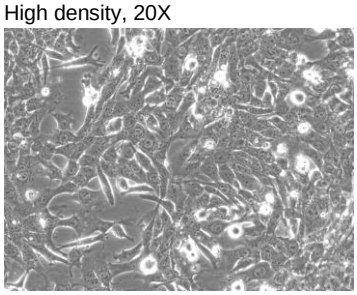
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
^{125}I Uptake	Pass QC
Fluorescence expression	Pass QC

Morphology:

Low density, 20X

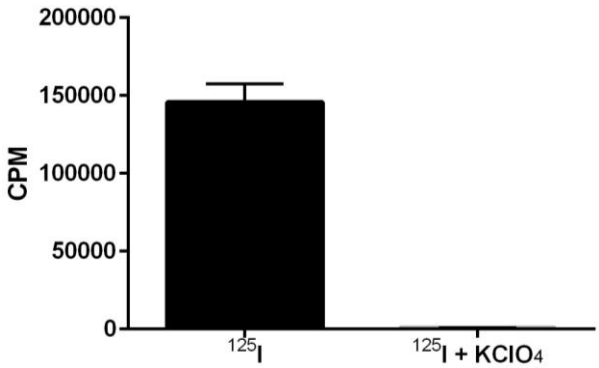


High density, 20X



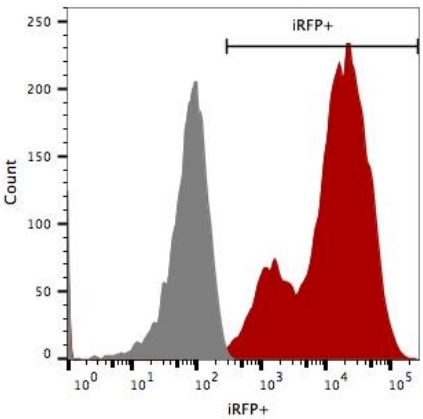
Low and high density photos taken 24 and 72 hours after thawing, respectively.

^{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.

Fluorescence Expression



4T1-mNIS-Neo/iRFP-Puro (red) or isotype control (4T1-Fluc-Neo; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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