B16F10-mNIS

Product Description

Product Name: B16F10-mNIS
Catalog Number: CL118
Lot Number: CL-IM118

Species: Mouse (*Mus musculus*)
Strain: C57BL/6
Cell type: Melanoma
Parental cells: B16F10 (ATCC® CRL-6475™)*
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Murine sodium iodide symporter (mNIS)

This is a monoclonal population derived from the melanoma B16F10 cell line (ATCC® CRL-6475™). Parental B16F10 cells were transduced with LV-mNIS (Imanis #LV008) encoding the murine sodium iodide symporter (mNIS) cDNA under the spleen focus-forming virus (SFFV) promoter. A monoclonal population was isolated following two rounds of selection in a methylcellulose-based semi-solid medium. The lentiviral vector is a self-inactivating (SIN) vector 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

The B16F10-mNIS cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental B16F10 cell line was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

Recommended Uses

B16F10-mNIS cells are suitable for *in vitro* and *in vivo* experimentation.

The mNIS transgene facilitates high-resolution, 3D SPECT/PET imaging of implanted cells. The mNIS protein in not immunogenic in mice. Therefore, these cells can be used for longitudinal imaging studies in both immunocompromised mice and immunocompetent C57BL/6 mice.

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

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 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. After 48 hours, replace the culture supernatant with complete growth medium and passage if needed. Cells should reach full confluency 2-3 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 37ºC until cells have dissociated (approx. 2-3 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. If the growth medium begins to turn brown before cells are ready to be passaged, replace with fresh medium.

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 supplemented with 5-10% DMSO.

Additional Considerations

B16F10 cells produce melanin, which is toxic to the cells. If the culture medium turns brown or black it should be immediately replaced with fresh culture medium even if the cells are not ready for passage.

References

[1]Miyoshi et al. J Virol. 1998. 72:8150-8157.

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Certificate of Analysis

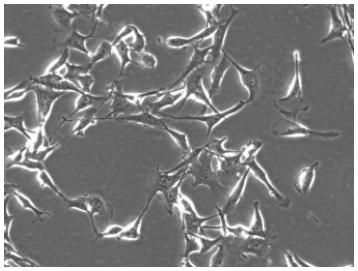
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	99%
Cells/vial	~ 4 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
NIS expression	Pass QC
Average Doubling Time	21.0 hours*

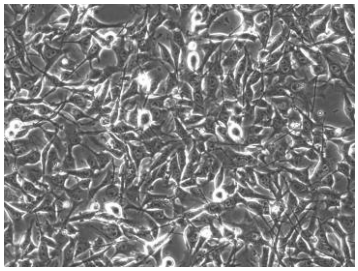
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:

Low density, 200X

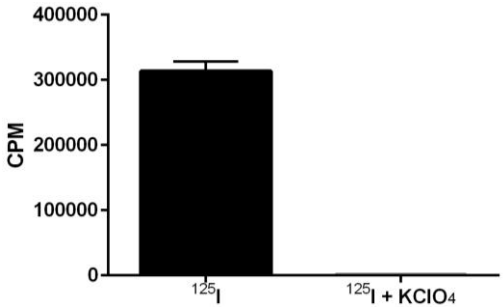


High density, 200X



Low and high density photos taken 24 and 44 hours after thawing, respectively.

NIS Expression:



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

Quality control by: CDL
Quality Assurance by: RLV
Effective Date: 26-Oct-2022

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