

## Product Description

Product Name: 4T1-mNIS  
Catalog Number: CL117  
Lot Number: CL-IM123

Species: Mouse (*Mus musculus*)  
Strain: BALB/cfC3H  
Cell type: Mammary carcinoma  
Parental cells: 4T1 (ATCC® CRL-2539™)\*  
Morphology: Epithelial  
Growth mode: Adherent  
Reporter gene: Murine sodium iodide symporter (mNIS)

This is a monoclonal population derived from the mammary carcinoma 4T1 cell line (ATCC® CRL-2539™). Parental 4T1 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 three 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<sup>1</sup>.

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## Mycoplasma Testing

The 4T1-mNIS cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

## Cell Line Authentication

The parental 4T1 cell line was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

## Recommended Uses

4T1-mNIS cells are suitable for *in vitro* and *in vivo* experimentation.

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

## References

<sup>1</sup>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 -130°C).

## Complete Growth Medium

RPMI-1640 Medium (RPMI)  
10% fetal bovine serum (FBS)  
1% Penicillin/Streptomycin

## 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. 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<sub>2</sub>. After 48 hours, replace the culture supernatant with complete growth medium and passage cells if needed. Cells should reach full confluency 3-4 days after thawing.

## Subculturing Instructions

Volumes are given for a T75 flask; increase or decrease as needed. 4T1 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<sub>2</sub> incubator.

4T1 cells frequently clump and should be passaged when they reach 80-90% confluency overall. For maintenance, a subcultivation ratio of 1:10 is recommended. At this ratio cells will be ready for passage every 3-4 days. Cell clumping is especially common after initial thawing and cells may need to be passaged more frequently at a lower subcultivation ratio for 2-3 passages after thawing.

## Freezing Medium

4T1-mNIS 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.

## Certificate of Analysis

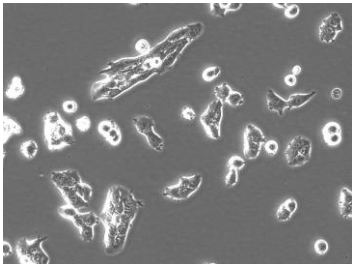
Testing performed by Imanis Life Sciences

| Test description               | Result                    |
|--------------------------------|---------------------------|
| Post thaw viable cell recovery | 99%                       |
| Cells/vial                     | ~ 4 x 10 <sup>6</sup>     |
| Sterility                      | No contamination detected |
| Mycoplasma                     | No contamination detected |
| NIS expression                 | Pass QC                   |
| Average Doubling Time          | 14.3 hours*               |

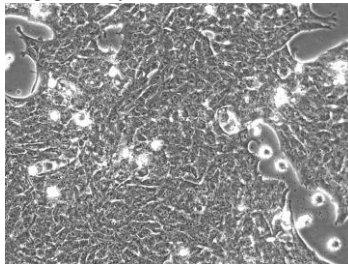
\*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

## Morphology:

Low density, 200X

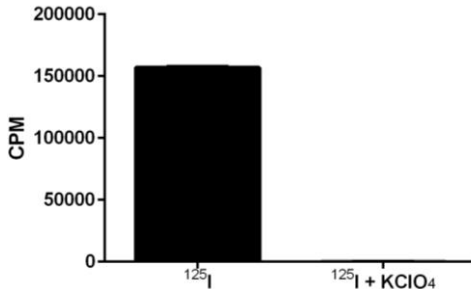


High density, 200X



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

## NIS Expression:



Uptake of <sup>125</sup>I by 3 x 10<sup>5</sup> cells was assayed in the presence or absence of KClO<sub>4</sub>, an inhibitor of NIS-mediated <sup>125</sup>I uptake.

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