

Product Description

Product Name: CT26.WT-mNIS-Neo/eGFP-Puro
 Catalog Number: CL045
 Lot Number: CL-IM50
 Species: Mouse (*Mus musculus*)
 Strain: BALB/c
 Cell type: Colorectal carcinoma
 Parental cells: CT26 (ATCC® CRL-2638™)*
 Morphology: Epithelial
 Growth mode: Adherent
 Reporter genes: Murine sodium iodide symporter (mNIS)
 Enhanced green fluorescent protein (eGFP)
 Selection genes: Neomycin (Neo)
 Puromycin (Puro)

CT26.WT-mNIS-Neo/eGFP-Puro is a polyclonal population of the murine colorectal cancer CT26.WT cell line transduced with lentiviral vectors encoding 1) the murine sodium iodide symporter (mNIS) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin (Neo) resistance gene via a P2A cleavage peptide (Imanis #LV025), and 2) the enhanced green fluorescent protein (eGFP) cDNA under the SFFV promoter linked and the puromycin (Puro) resistance gene under the phosphoglycerate kinase (PGK) promoter (Imanis #LV1031). 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 LTR¹.

* The ATCC trademark and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection

Mycoplasma Testing

This cell line has tested negative for mycoplasma contamination.

Cell line Authentication

Authentication of the parental CT26.WT cell line was confirmed by short tandem repeat (STR) profiling.

Recommended Uses

These cells are suitable for *in vitro* and *in vivo* experimentation.

The mNIS 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

¹Miyoshi 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

Dulbecco's Modified Eagle's Medium (DMEM)
 10% Fetal bovine serum (FBS)
 1% Penicillin/Streptomycin
 0.4 mg/mL G418
 3 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 37°C 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% CO₂. 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 (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% CO₂ incubator.

For maintenance, a subcultivation ratio of 1:10 is recommended. At this ratio cells will be ready for passage approximately every 3-4 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 easily detach from tissue culture surfaces. Coating culture pates with poly-D-Lysine prior to use is recommended for applications involving frequent washing or rocking. These cells will not form 100% confluent monolayers; the cells are ready for passage when they reach 80-90% confluency.

CT26.WT-mNIS-Neo/eGFP-Puro

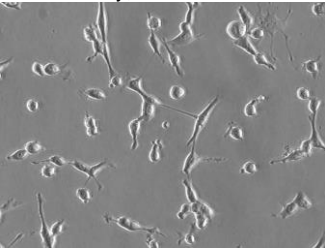
Certificate of Analysis

Testing performed by Imanis Life Sciences:

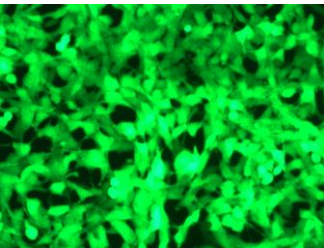
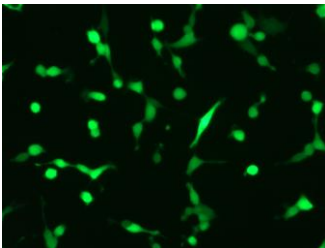
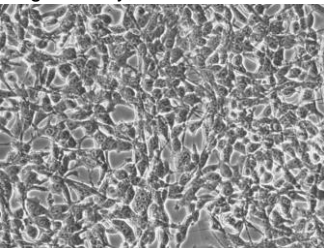
| Test description | Result |
|--------------------------------|---------------------------|
| Post thaw viable cell recovery | Pass QC |
| Sterility | No contamination detected |
| Mycoplasma | No contamination detected |
| Neomycin selection | Pass QC |
| Puromycin selection | Pass QC |
| ¹²⁵ I Uptake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

Morphology:

Low density, 200X

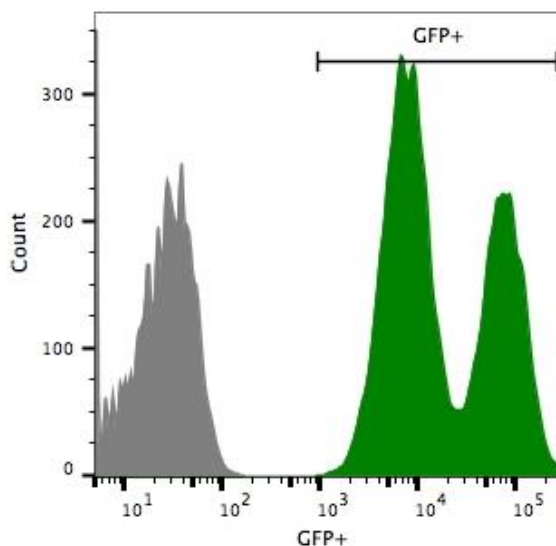


High density, 200X



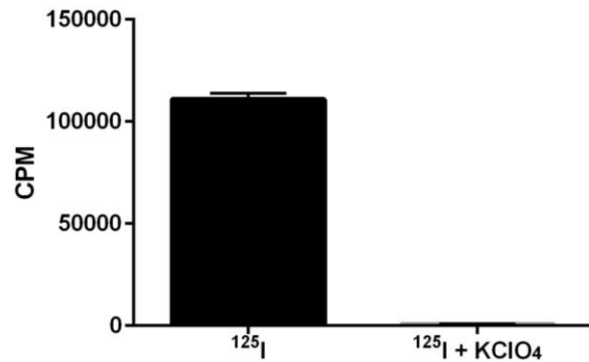
Low and high density photos taken at various times after thawing.

Fluorescence Expression:



CT26.WT-mNIS-Neo/eGFP-Puro (green) or isotype control (CT26.WT-mNIS-Neo; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

¹²⁵I Uptake:



Uptake of ¹²⁵I by 3×10^6 cells was assayed in the presence or absence of KClO₄, an inhibitor of NIS-mediated ¹²⁵I uptake.

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Quality Control by: RLV

Quality Assurance by: SPR

Effective Date: 10/30/15