

## Product Description

Product Name: CT26.WT-iRFP-Neo/mNIS-Puro  
 Catalog Number: CL093  
 Lot Number: CL-IM90

Species: Mouse (*Mus musculus*)  
 Strain: BALB/c  
 Cell type: Colorectal carcinoma  
 Parental cells: CT26.WT (ATCC® CRL-2638™)  
 Morphology: Epithelial  
 Growth mode: Adherent  
 Reporter genes: Near infrared fluorescent protein (iRFP)  
 Murine sodium iodide symporter (mNIS)  
 Selection genes: Neomycin (Neo)  
 Puromycin (Puro)

This is a polyclonal population derived from the murine colorectal carcinoma CT26.WT cell line (ATCC® CRL-2638™). Parental CT26.WT cells were transduced with 1) LV-iRFP-P2A-Neo (Imanis #LV033) encoding the near infrared fluorescent protein (iRFP; ex/em = 690/710 nm) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene (Neo) via a P2A cleavage peptide and 2) LV-mNIS-PGK-Puro (Imanis #LV022) encoding the murine sodium iodide symporter (mNIS) cDNA under the SFFV promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter. 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<sup>1</sup>.

## Mycoplasma Testing

The CT26.WT-iRFP-Neo/mNIS-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,<sup>2,3</sup> 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<sup>4,5</sup>. The parental CT26.WT cell line used to generate CT26.WT-iRFP-Neo/mNIS-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

## Recommended Uses

CT26.WT-iRFP-Neo/mNIS-Puro cells are suitable for *in vitro* and *in vivo* experimentation. CT26.WT cells form tumors and pulmonary metastases post implantation into syngenic BALB/c mice<sup>6</sup>. The iRFP and mNIS transgenes facilitate *in vivo* noninvasive fluorescent and high-resolution 3D PET/SPECT imaging, respectively, of implanted cells.

## References

- <sup>1</sup>Miyoshi et al. J Virol. 1998. 72:8150-8157.
- <sup>2</sup>Hughes et al. BioTechniques 2007. 43: 575-586.
- <sup>3</sup>Chatterjee et al. Science 2007. 315:928-931.
- <sup>4</sup><https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html>
- <sup>5</sup><http://www.aacrjournals.org/site/InstrAuthors/fora.xhtml#celllineuse>
- <sup>6</sup>Wang et al. J Immunol, 1995. 154:4685-4692.

## 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 (to maintain high iRFP expression)  
 3 µg/mL puromycin (to maintain high mNIS 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<sub>2</sub>. After 48 hours, replace the culture supernatant with complete growth medium containing 0.4 mg/mL G418 and 3 µg/mL puromycin. Cells should reach full confluency 2-3 days after thawing.

## Subculturing Instructions

Volumes are given for a T75 flask. Increase or decrease as needed. In order to maintain high iRFP and mNIS expression, it is recommended that cells be subcultured in the presence of 0.4 mg/mL G418 and 3 µg/mL puromycin. CT26.WT-iRFP-Neo/mNIS-Puro cells do not form a 100% confluent monolayer. It is recommended that cells be passaged when they reach ~80% confluency.

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. 1-2 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.

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

CT26.WT-iRFP-Neo/mNIS-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.

## Additional Considerations

CT26.WT-iRFP-Neo/mNIS-Puro cells easily detach from tissue culture surfaces. Coating culture plates with poly-D-Lysine prior to use can be used to increase cell adherence to the plates if necessary.

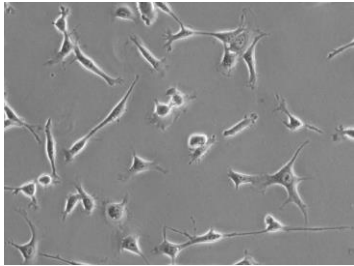
## Certificate of Analysis

Testing performed by Imanis Life Sciences

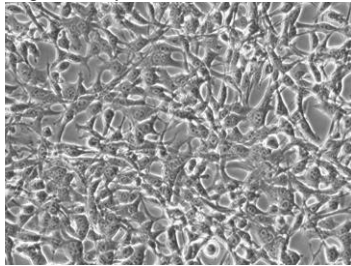
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Fluorescence expression	Pass QC
<sup>125</sup> I uptake	Pass QC

## Morphology:

Low density, 20X

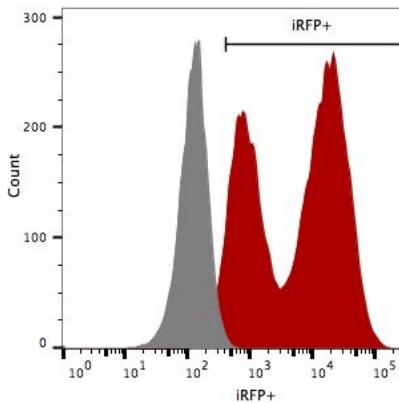


High density, 20X



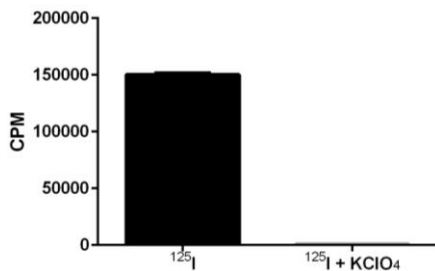
Low and high density photos taken 24 and 72 hours after thawing, respectively.

## Fluorescence Expression:



CT26.WT-iRFP-Neo/mNIS-Puro cells (red) or isotype control (CT26.WT-Fluc-Neo; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry (20,000 events).

## <sup>125</sup>I Uptake:



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

## Legal Disclaimers

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