CT26.WT-Fluc-Neo/iRFP-Puro

Product Description

Product Name: CT26.WT-Fluc-Neo/iRFP-Puro
Catalog Number: CL092
Lot Number: CL-IM89

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: Firefly luciferase (Fluc)
Near infrared fluorescent protein (iRFP)
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-Fluc-P2A-Neo (Imanis #LV011) encoding the firefly luciferase (Fluc) 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 = 690/710 nm) cDNA under the SFFV promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High Fluc 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 CT26.WT-Fluc-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 CT26.WT cell line used to generate CT26.WT-Fluc-Neo/iRFP-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

Recommended Uses

CT26.WT-Fluc-Neo/iRFP-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[6]. The Fluc and iRFP transgenes in the CT26.WT-Fluc-Neo/iRFP-Puro cells facilitate *in vivo* noninvasive imaging of implanted cells.

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]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 Fluc expression)
3 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 0.4 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 Fluc and iRFP expression, it is recommended that cells be subcultured in the presence of 0.4 mg/mL G418 and 3 µg/mL puromycin. CT26.WT-Fluc-Neo/iRFP-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_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

CT26.WT-Fluc-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.

Additional Considerations

CT26.WT-Fluc-Neo/iRFP-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.

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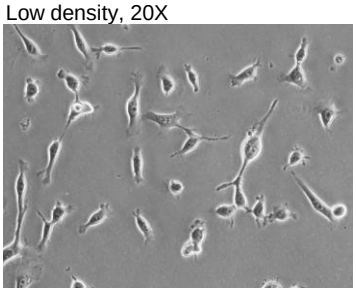
Certificate of Analysis

Testing performed by Imanis Life Sciences

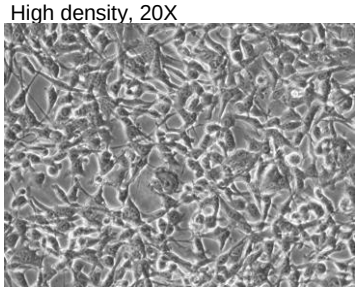
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC

Morphology:

Low density, 20X

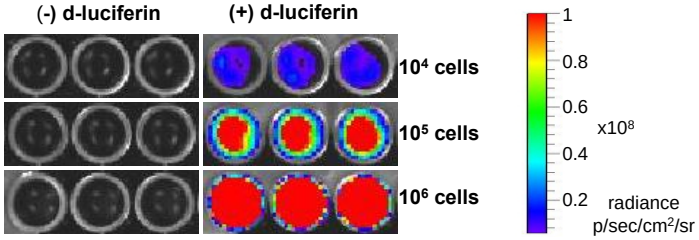


High density, 20X



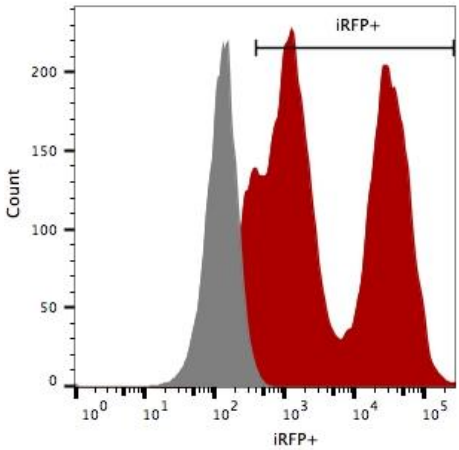
Low and high density photos taken 24 and 72 hours after thawing, respectively.

Luciferase Expression



10^4, 10^5, or 10^6 cells were placed in wells of a 96-well plate and 0.3 mg of d-luciferin was added to the indicated wells. The plate was immediately imaged using a Xenogen IVIS Spectrum.

Fluorescence Expression



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

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