

Product Description

Product Name: CT26.WT-Fluc-Neo
 Catalog Number: CL043
 Lot Number: IMP023

 Species: Mouse (*Mus musculus*)
 Strain: BALB/c
 Cell type: Colorectal carcinoma
 Parental cells: CT26.WT (ATCC® CRL-2638™)*
 Morphology: Epithelial
 Growth mode: Adherent
 Reporter gene: Firefly luciferase (Fluc)
 Selection gene: Neomycin (Neo)

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 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. High Fluc expressing cells were selected using G418. 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 been tested for mycoplasma contamination and is mycoplasma free.

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 Fluc transgene facilitates *in vivo* noninvasive bioluminescent imaging of implanted cells. Fluc 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.

Biosafety Notice

These cell lines were generated by transduction with a lentiviral vector. Cell lines transduced with lentiviral vectors are classified as biosafety level 2 reagents and should be used under appropriate biosafety level for institutional guidelines.

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

G418 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 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 (about 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 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 complete growth medium.
5. Incubate the culture at 37°C with 5% CO₂. Cells should reach full confluency 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 room temperature until cells have dissociated.
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

These cells can be amplified and used to generate additional frozen stocks. Cryopreservation of low passage stocks is recommended. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium without G418 supplemented with 5-10% DMSO.

Additional Considerations

CT26.WT 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

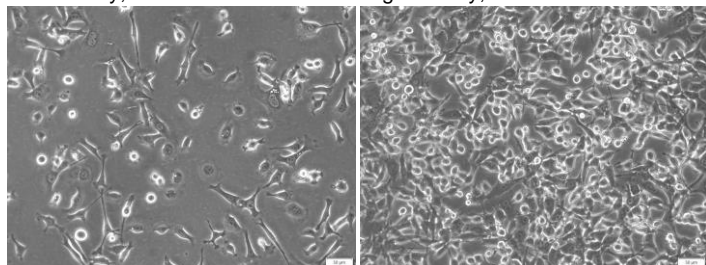
Test description	Result
Post thaw viable cell recovery	97%
Viable cells per vial	~ 9 x 10 ⁶
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Luciferase expression	Pass QC
Average doubling time	14.98 hours*

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

Morphology

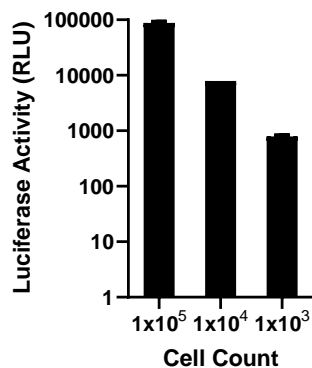
Low density, 200X

High density, 200X



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

Luciferase Expression



The indicated number of cells were placed in wells of a 96-well plate. After the addition of 15 mg/mL d-luciferin, bioluminescence was immediately imaged using a microplate reader.

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Quality control by: AWD
 Quality Assurance by: RLV
 Effective Date: 26-Jul-2023