4T1-Fluc-Neo/eGFP-Puro

Product Description

Product Name: 4T1-Fluc-Neo/eGFP-Puro
Catalog Number: CL023
Lot Number: CL-IM166

Species: Mouse (*Mus musculus*)
Strain: BALB/cfC3H
Cell type: Mammary carcinoma
Parental cells: 4T1 (ATCC® CRL-2539™)*
Morphology: Epithelial
Growth mode: Adherent
Reporter genes: Firefly luciferase (Fluc)
Enhanced green fluorescent protein (eGFP)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

This is a polyclonal population derived from the mammary carcinoma 4T1 cell line (ATCC® CRL-2539™). Parental 4T1 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 and linked to the neomycin resistance gene (Neo) via a P2A cleavage peptide, and 2) LV-eGFP-PGK-Puro (Imanis #LV031) encoding the enhanced green fluorescent protein (eGFP) cDNA under the SFFV promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter. High Fluc and eGFP 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].

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Mycoplasma Testing

This cell line has been tested for mycoplasma contamination and is mycoplasma free.

Cell line Authentication

Authentication of the parental 4T1 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. eGFP is not recommended for whole animal in-live imaging. Rather, samples can be collected post mortem for analysis by conventional fluorescence microscopy or flow cytometry.

Fluc and eGFP are immunogenic and may cause tumor rejection in immunocompetent mice. For the most consistent results, immunocompromised mice are recommended for studies.

References

[1]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 -130ºC).

Complete Growth Medium

RPMI-1640 Medium (RPMI)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
0.1 mg/mL G418
2 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 37ºC 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 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% CO_2. Cells should reach full confluency 2-3 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 (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_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. 4T1 cells frequently clump and should be passaged when they reach 80-90% confluency overall. 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

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.

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Certificate of Analysis

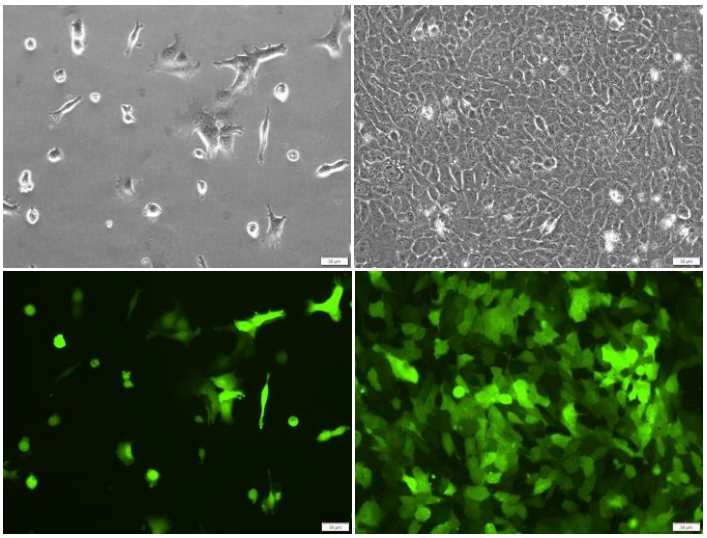
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	100% viability
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC
Average doubling time	13.8 h*

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

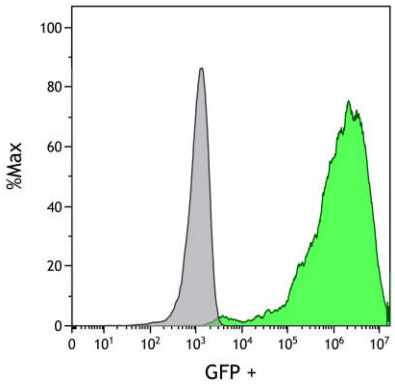
Morphology

Low density, 200X High density, 200X



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

Fluorescence Expression



4T1-Fluc-Neo/eGFP-Puro (green) or isotype control (4T1; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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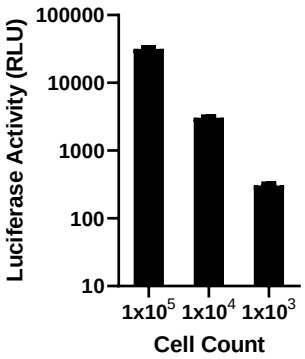
Luciferase Expression:



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 read using a microplate reader.

Quality control by: AWD
Quality Assurance by: RLV
Effective Date: 16-Nov-2022