4T1-Fluc-Neo (monoclonal)

Product Description

Product Name: 4T1-Fluc-Neo (monoclonal)
Catalog Number: CL126
Lot Number: CL-IM213

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

This is a monoclonal population derived from the mammary carcinoma 4T1 cell line (ATCC® CRL-2539™). Parental 4T1 cells were transduced with LV-Luc2-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) cDNA via a P2A cleavage peptide. A monoclonal population was isolated following selection with G418 and two rounds of selection in a methylcellulose-based semi-solid medium. The lentiviral vector is a self-inactivating (SIN) vector 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].

* 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 certified mycoplasma free.

Cell Line Authentication

The parental 4T1 cell line was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

Recommended Uses

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

The Fluc transgene in the 4T1-Fluc-Neo cells 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

[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 -130°C).

Complete Growth Medium

RPMI-1640 Medium (RPMI)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin

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 (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 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. After 48 hours, replace the culture supernatant with complete growth medium and passage cells if needed. Cells should reach full confluency 2-3 days after thawing.

Subculturing Instructions

Volumes are given for a T75 flask; increase or decrease as needed. To maintain high Fluc expression, cells can be subcultured in the presence of 0.1 mg/mL G418.

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. 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 approximately every 3 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

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 supplemented with 5-10% DMSO.

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Certificate of Analysis

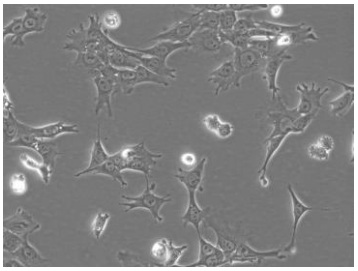
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	100%
Cell per vial	~5 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Luciferase expression	Pass QC
Average doubling time	11.9 hours*

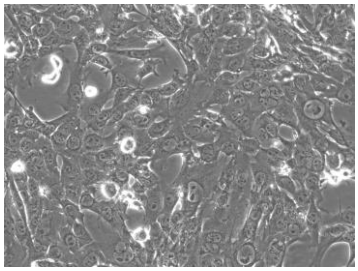
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:

Low density, 200X

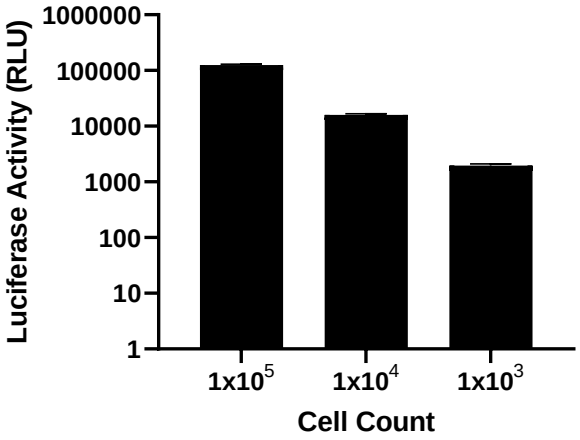


High density, 200X



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 1.5 mg/mL d-luciferin, bioluminescence was immediately read using a microplate reader.

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