4T1-eGFP-Puro

Product Description

Product Name: 4T1-eGFP-Puro
Catalog Number: CL021
Lot Number: CL-IM154

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

This is a polyclonal population derived from the mammary carcinoma 4T1 cell line (ATCC® CRL-2539™). Parental 4T1 cells were transduced with LV-eGFP-PGK-Puro (Imanis #LV031) encoding the enhanced green fluorescent protein (eGFP) cDNA under the spleen focus-forming virus (SFFV) promoter and the puromycin resistance gene (Puro) cDNA under the phosphoglycerate kinase (PGK) promoter. High eGFP-expressing cells were selected using puromycin. 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].

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Mycoplasma Testing

This cell line has tested negative for mycoplasma contamination.

Cell line Authentication

Authentication of the parental 4T1 cell line was confirmed by short tandem repeat (STR) profiling.

Recommended Uses

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.

eGFP 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 -130ºC).

Complete Growth Medium

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

Caution! Typical commercial puromycin stocks are provided at a concentration of 10 mg/mL or 10,000X.

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 pre-warmed 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 pre-warmed complete growth medium.
5. Incubate the culture at 37°C with 5% CO_2. Cells should reach full confluency 1-2 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 approximately every 3 days.

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 puromycin supplemented with 5-10% DMSO.

Additional Considerations

Over trypsinization of 4T1 cells can damage the cells. During trypsinization the cells should be monitored carefully, and the trypsin neutralized immediately upon cell detachment.

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Certificate of Analysis

Testing performed by Imanis Life Sciences

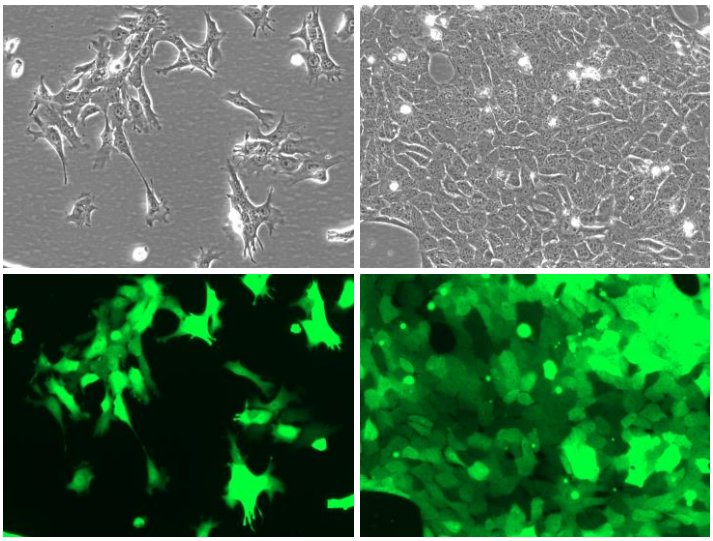
Test description	Result
Post thaw viable cell recovery	99%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC
Average doubling time	12.3 hours*

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

Morphology:

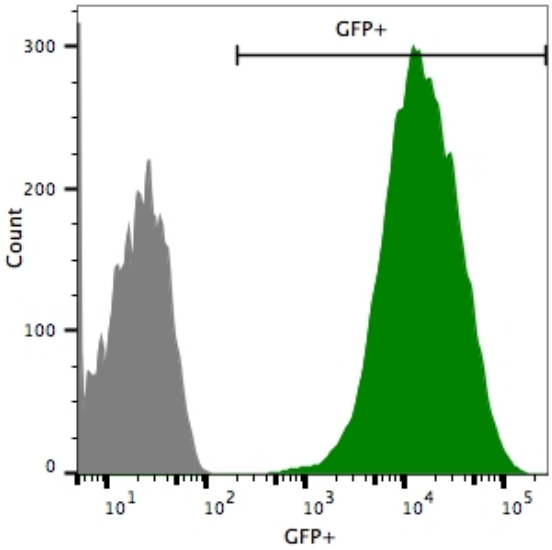
Low density, 200X

High density, 200X



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

Fluorescence Expression:



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

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