CT26.WT-eGFP-Puro

Product Description

Product Name: CT26.WT-eGFP-Puro
Catalog Number: CL044
Lot Number: CL-IM218

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: Enhanced green fluorescent protein (eGFP)
Selection gene: 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 LV-SFFV-eGFP-PGK-Puro (Imanis #LV031) encoding the Enhanced green fluorescent protein (GFP) cDNA under the spleen focus-forming virus (SFFV) promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter. High GFP 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].

* The ATCC trademark and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection

Mycoplasma Testing

This cell line has tested negative for mycoplasma contamination.

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.

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

Dulbecco's Modified Eagle's Medium (DMEM)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
3 µg/mL Puromycin (Puro)

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 Puromycin to the growth medium.

Caution! Most commercial stocks of puromycin are supplied at 10,000X concentration.

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 ~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.
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 3-4 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 Puromycin supplemented with 5-10% DMSO.

Additional Considerations

CT26.WT-eGFP-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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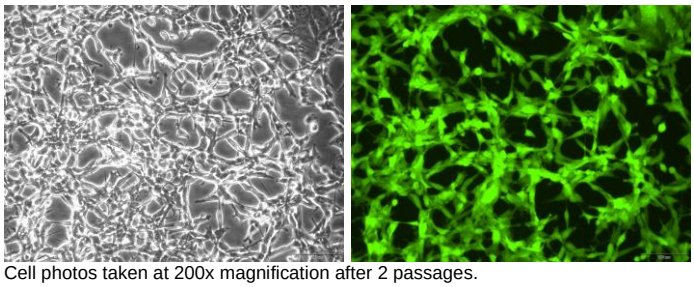
Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	Pass QC
Cells/Vial	~ 4 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC
Average doubling time	19.4 hours*

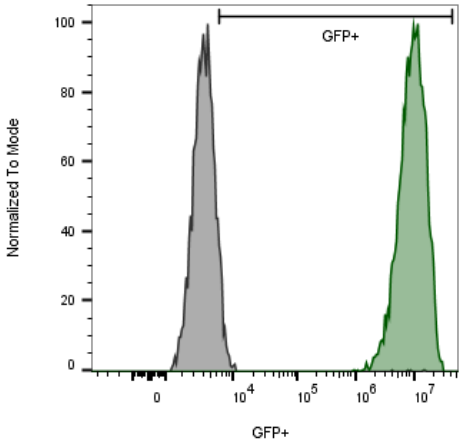
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology



Cell photos taken at 200x magnification after 2 passages.

Fluorescence Expression



CT26.WT-eGFP-Puro (green) or isotype control (CT26.WT-Fluc-Neo, grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

Legal Disclaimers

Quality control by: AMW
Quality Assurance by: RLV
Effective Date: 17-Nov-2021