A20-eGFP-Neo/Fluc-Puro

Product Description

Product Name: A20-eGFP-Neo/Fluc-Puro
Catalog Number: CL152
Lot Number: CL-IM226

Species: Mouse (*Mus musculus*)
Strain: Balb/c
Cell type: Lymphoma
Parental cells: A20 (ATCC® TIB-208™)*
Morphology: Lymphoblast
Growth mode: Suspension
Reporter genes: Enhanced green fluorescent protein (eGFP)
Firefly luciferase (Fluc)
Selection genes: Neomycin (Neo)
Puromycin (Puro)

This is a polyclonal cell line derived from the murine lymphoma A20 cell line (ATCC® TIB-208™). Parental A20 cells were transduced with, 1) LV-eGFP-P2A-Neo (Imanis #LV067) encoding the enhanced green fluorescent (eGFP) 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-Fluc-P2A-Puro (Imanis #LV012) encoding the firefly luciferase (Fluc) cDNA under the SFFV promoter and linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. A high Fluc and eGFP expressing population was generated by selection using puromycin and G418 followed by selection using a methylcellulose based semi-solid medium. 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].

* 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 A20 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.

Biosafety Notice

This cell line was 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

RPMI-1640 Medium (RPMI)
50 μM β-mercaptoethanol
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
1 mg/mL G418
1 μg/mL Puromycin

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.

Caution! Typical commercial puromycin stocks are provided at a concentration of 10 mg/mL or 10,000X.
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 (~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 ~300 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in complete growth medium to a final density of 1 x 10^6 cells/mL. Transfer the cells to a T25 or T75 suspension culture flask.
5. Incubate the culture at 37°C with 5% CO_2.

Subculturing Instructions

Passage cells by dilution in fresh complete growth medium. If desired, use centrifugation to remove excess debris:

1. Pipet the cell suspension gently to dislodge any cells loosely attached to the culture flask. Transfer the desired volume (half, one fourth, etc.) of the cells to a conical tube.
2. Centrifuge at ~150 x g for 3 min. (Note: a short, low speed spin is recommended to limit the amount of cell debris in the pellet.)
3. Remove supernatant and resuspend cells in complete growth medium. Transfer to an appropriate sized flask.

The cells should be subcultured as needed to maintain a density between 5 x 10^5 and 3 x 10^6 cells/mL.

Note: These cells have a tendency to clump; if cell clumps become too large, cells in the center of the clumps will begin to die. More frequent passaging of the cells at lower subcultivation ratios and with repeated gentle pipetting can help reduce clumping.

Freezing Medium

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

References

[1]Miyoshi et al. J Virol. 1998. 72:8150-8157.

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Certificate of Analysis

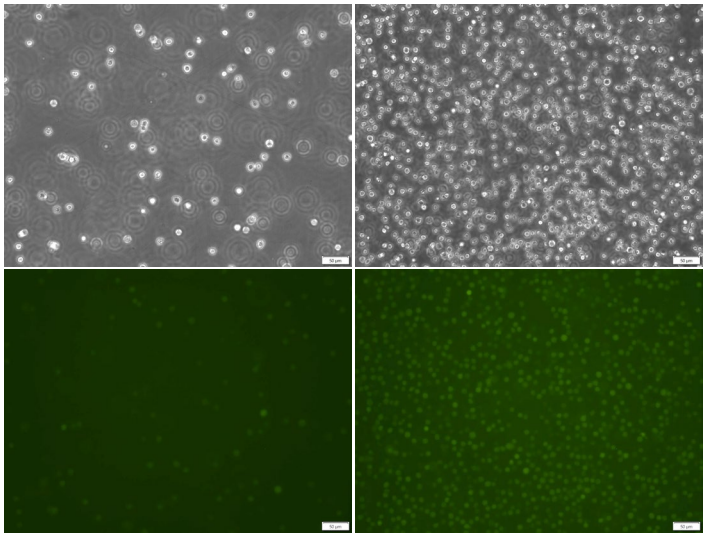
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	82%
Viable cells per vial	~ 1.2 x 10^7
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
eGFP expression	Pass QC
Luciferase expression	Pass QC
Average doubling time	12.9 h*

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

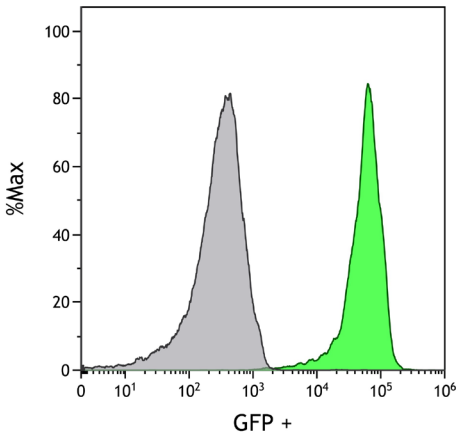
Morphology

Low density, 200X High density, 200X



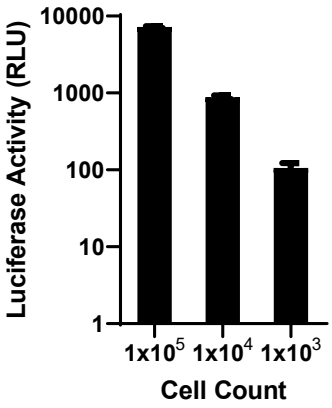
Low- and high-density photos taken at various times after thawing.

Fluorescence Expression



A20-eGFP-Neo/Fluc-Puro (green) or isotype control (A20-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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: 17-May-2023

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