A20-Fluc-Puro

Product Description

Product Name: A20-Fluc-Puro
Catalog Number: CL147
Lot Number: CL-IM221

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

This is a cell line derived from the murine lymphoma A20 cell line (ATCC® TIB-208™). Parental A20 cells were transduced with LV-Fluc-P2A-Puro (Imanis #LV012) encoding the firefly luciferase (Fluc) cDNA under the spleen focus-forming virus (SFFV) promoter and linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. A high Fluc expressing population was generated by selection using puromycin followed by selection using 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].

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Mycoplasma Testing

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

Cell Line Authentication

The parental A20 cell line was authenticated and certified free of interspecies cross contamination by 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. 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 -130ºC).

Complete Growth Medium

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

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! 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 (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 ~200 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium. Remove an aliquot for counting.
5. Dilute the cells further with growth medium to achieve a final density between 1 and 2 x 10^6 cells/mL. Transfer the cells to a T25 or T75 flask based on volume.
6. Incubate the culture at 37°C with 5% CO_2.

Subculturing Instructions

The cells should be subcultured as needed to maintain a density between 5 x 10^5 and 3 x 10^6 cells/mL. The cells can be passaged by dilution in fresh complete growth medium. Occasional passage using centrifugation as described below is recommended to limit the amount of debris in cultures.

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.

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. Preparation of low passage frozen stocks is highly recommended. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium without antibiotics supplemented with 5-10% DMSO.

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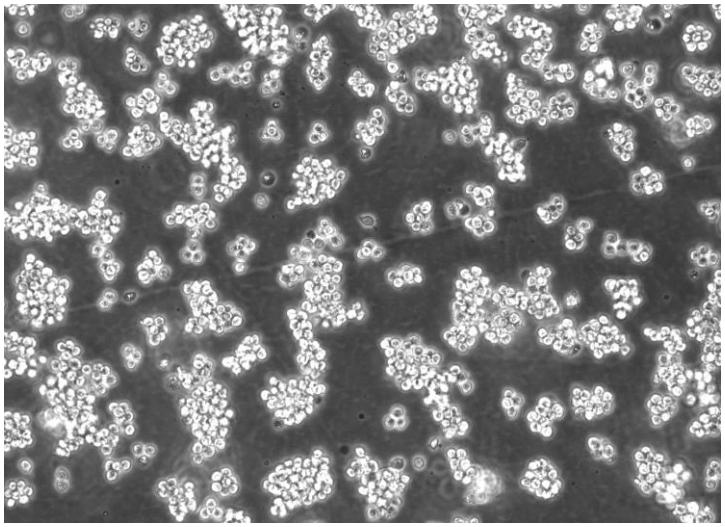
Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	98%
Cells per vial	~ 8 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Average doubling time	17.6 h*

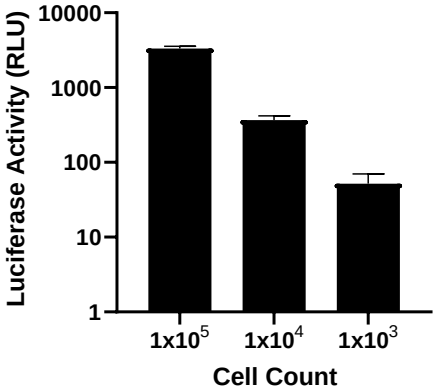
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology



Cell photo taken at 200x magnification.

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.

Quality control by: RLV
Quality Assurance by: LAS
Effective Date: 10-May-2022

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