

## Product Description

Product Name: C1498-Fluc-Puro  
Catalog Number: CL144  
Lot Number: CL-IM155

Species: Mouse (*Mus musculus*)  
Strain: C57BL/6J  
Cell type: Lymphoblast (acute myeloid leukemia; AML)  
Parental cells: C1498 (ATCC® TIB-49™)\*  
Morphology: Lymphoblast  
Growth mode: Suspension  
Reporter gene: Firefly luciferase (Fluc)  
Selection gene: Puromycin (Puro)

This is a cell line derived from the murine acute myeloid leukemia C1498 cell line (ATCC® TIB-49™). Parental C1498 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<sup>1</sup>.

\* 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 C1498 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

<sup>1</sup>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

High glucose DMEM  
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 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 ~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<sup>6</sup> cells/mL. Transfer the cells to a T25 or T75 flask based on volume.
6. Incubate the culture at 37°C with 5% CO<sub>2</sub>.

## Subculturing Instructions

The cells should be subcultured as needed to maintain a density between 5 x 10<sup>5</sup> and 2 x 10<sup>6</sup> 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 fifth, 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.

## 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.

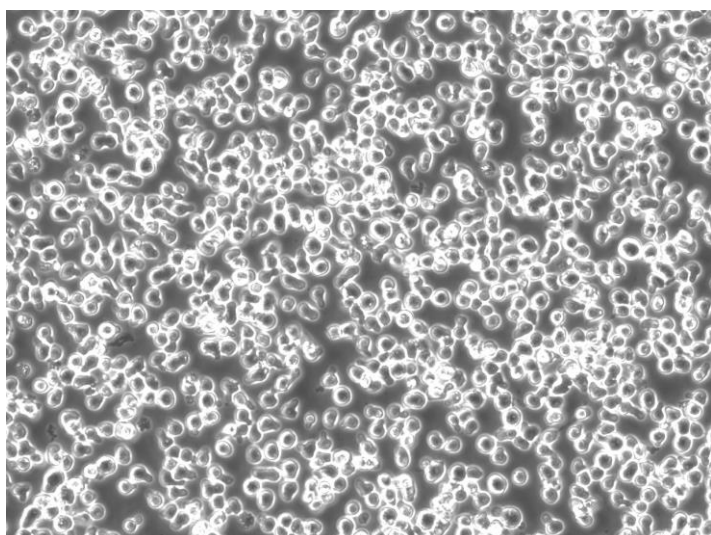
## Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	82%
Cells per vial	$\sim 7 \times 10^6$
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Average doubling time	29.3 h*

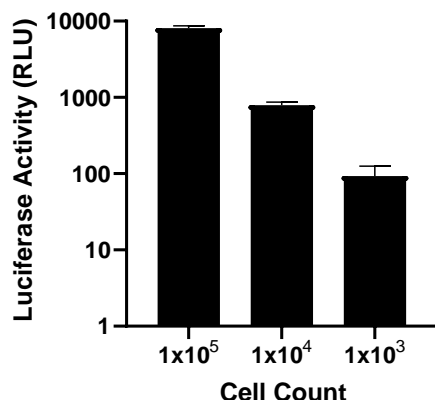
\*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

## 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.

## Legal Disclaimers

### LIMITED PRODUCT WARRANTY

THIS WARRANTY LIMITS OUR LIABILITY TO REPLACEMENT OF THIS PRODUCT. NO OTHER WARRANTIES OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, ARE PROVIDED BY IMANIS. IMANIS SHALL HAVE NO LIABILITY FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL, OR INCIDENTAL DAMAGES ARISING OUT OF THE USE, THE RESULTS OF USE, OR THE INABILITY TO USE THIS PRODUCT.

FOR *IN VITRO* USE ONLY. THIS CERTIFICATE IS A DECLARATION OF ANALYSIS AT THE TIME OF MANUFACTURE.

### PURCHASER NOTIFICATION

### LIMITED LICENSE NOTICE – RESEARCH USE ONLY

THE IMANIS CELL LINES ARE NOT INTENDED FOR USE IN HUMANS. CELL LINES TRANSDUCED WITH LENTIVIRAL VECTORS ARE CLASSIFIED AS BIOSAFETY LEVEL 2 REAGENTS AND SHOULD BE USED UNDER THE APPROPRIATE BIOSAFETY LEVEL PER INSTITUTIONAL GUIDELINES.

THE PURCHASER AGREES THAT IMANIS MATERIALS DESIGNATED AS BIO-SAFETY LEVEL 2 CONSTITUTE KNOWN PATHOGENS AND THAT OTHER IMANIS MATERIALS NOT SO DESIGNATED AND ANY PROGENY OR MODIFICATION MAY BE PATHOGENIC UNDER CERTAIN CONDITIONS. PURCHASER ASSUMES ALL RISK AND RESPONSIBILITY IN CONNECTION WITH THE RECEIPT, HANDLING, STORAGE, DISPOSAL, TRANSFER AND USE OF THE IMANIS MATERIALS INCLUDING WITHOUT LIMITATION TAKING ALL APPROPRIATE SAFETY AND HANDLING PRECAUTIONS TO MINIMIZE HEALTH OR ENVIRONMENTAL RISK. PURCHASER AGREES THAT ANY ACTIVITY UNDERTAKEN WITH THE IMANIS MATERIALS AND ANY PROGENY OR MODIFICATION WILL BE CONDUCTED IN COMPLIANCE WITH ALL APPLICABLE GUIDELINES, LAWS AND REGULATIONS.

THE IMANIS MATERIAL, ANY OTHER IMANIS PRODUCTS, AND ANY TECHNICAL INFORMATION AND ASSISTANCE PROVIDED BY IMANIS ARE PROVIDED "AS IS", WITHOUT WARRANTIES OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, MANUFACTURE ACCORDING TO cGMP STANDARDS, TYPICALITY, SAFETY, ACCURACY AND NON-INFRINGEMENT.

IN NO EVENT SHALL IMANIS, ITS PARENTS, SUBSIDIARIES, DIRECTORS, OFFICERS, AGENTS, EMPLOYEES, ASSIGNS, SUCCESSORS AND AFFILIATE (COLLECTIVELY "IMANIS INDEMNIFIED PARTIES") BE LIABLE FOR INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES OF ANY KIND IN CONNECTION WITH OR ARISING OUT OF THIS AGREEMENT (WHETHER IN CONTRACT, TORT, NEGLIGENCE, STRICT LIABILITY, STATUTE OR OTHERWISE) EVEN IF IMANIS HAS BEEN ADVISED, KNEW OR SHOULD HAVE KNOWN OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING, BUT NOT LIMITED TO, LOST PROFITS, COST OF CAPITAL, COST OF SUBSTITUTE PRODUCTS OR CLAIMS OF LICENSEE'S CUSTOMERS FOR SUCH DAMAGE. IN NO EVENT SHALL IMANIS' CUMULATIVE LIABILITY EXCEED THE ACTUAL AMOUNTS PAID BY PURCHASER UNDER THIS AGREEMENT FOR THE TWELVE (12) MONTH PERIOD PRECEDING THE DATE OF THE EVENT GIVING RISE TO THE CLAIM. THE PROVISIONS OF THIS SECTION SHALL SURVIVE THE EXPIRATION OR TERMINATION OF THIS AGREEMENT AND SHALL APPLY EVEN IF THE LIMITED REMEDY SPECIFIED IN THIS AGREEMENT IS FOUND TO HAVE FAILED OF ITS ESSENTIAL PURPOSE.

Quality control by: LAS

Quality Assurance by: RLV

Effective Date: 20-Jul-2022