

# LL/2-eGFP-Puro

## Product Description

Product Name: LL/2-eGFP-Puro  
 Catalog Number: CL051  
 Lot Number: CL-IM179

Species: Mouse (*Mus musculus*)  
 Strain: C57BL  
 Cell type: Lewis lung carcinoma  
 Parental cells: LL/2 (ATCC® CRL-1642™)\*  
 Morphology: Epithelial  
 Growth mode: Loosely adherent  
 Reporter gene: Enhanced green fluorescent protein (eGFP)  
 Selection gene: Puromycin (Puro)

This is a polyclonal population derived from the Lewis lung carcinoma LL/2 cell line (ATCC® CRL-1642™). Parental LL/2 cells were transduced with a 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) under the phosphoglycerate kinase (PGK) promoter. High eGFP expressing cells were selected using puromycin. 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<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 tested negative for mycoplasma contamination.

## Cell line Authentication

Authentication of the parental LL/2 cell line was confirmed by short tandem repeat (STR) profiling.

## Recommended Uses

These cells are suitable for *in vitro* and *in vivo* experimentation.

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

<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

Dulbecco's Modified Eagle's Medium (DMEM)  
 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.

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.

## 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 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<sub>2</sub>. 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<sub>2</sub> incubator.

These cells should be passaged when they reach ~80% confluency. Beyond this density, the cells begin to lift off the plate or clump together in large balls. Some of these cells remain viable and can be collected by centrifugation and re-plated if needed. For maintenance, a subcultivation ratio of 1:10 is recommended. At this ratio cells will be ready for passage every 3-4 days.

## Freezing Medium

LL/2-eGFP-Puro 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

LL/2 cells are loosely adherent. Coating culture plates with poly-D-Lysine prior to use can be used to increase cell adherence to the plates if necessary.

## Certificate of Analysis

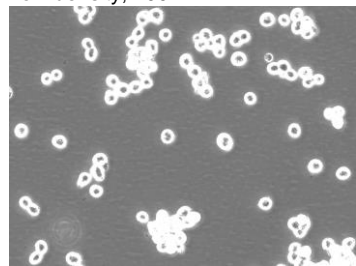
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	95%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC
Average doubling time	11.8 h*

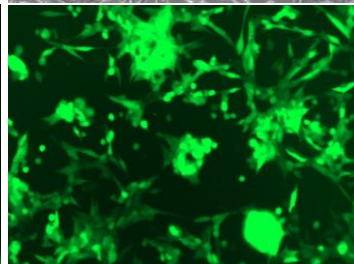
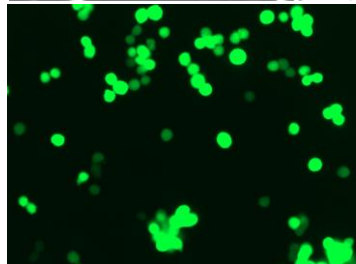
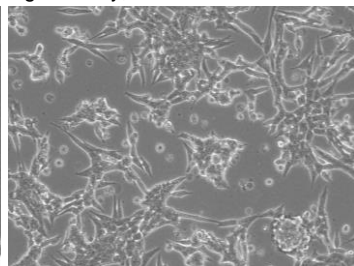
\*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

## Morphology:

Low density, 200X

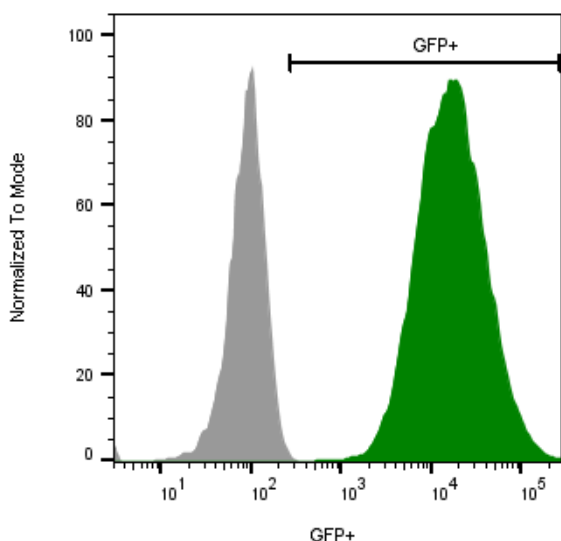


High density, 200X



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

## Fluorescence Expression



LL/2-eGFP-Puro (green) or isotype control (LL/2-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

## 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

IMANIS LIFE SCIENCES HAS A LIMITED LICENSE UNDER PATENTS OWNED BY THE SALK INSTITUTE FOR BIOLOGICAL STUDIES THAT PERMITS IMANIS LIFE SCIENCES TO SELL PRODUCTS CONTAINING WPRE FOR RESEARCH USE ONLY AND NOT FOR ANY COMMERCIAL USES. EXCLUDED COMMERCIAL USES INCLUDE WITHOUT LIMITATION MANUFACTURING, PROVIDING A SERVICE, THERAPEUTIC, DIAGNOSTIC AND PROPHYLACTIC USES, AND ANY OTHER COMMERCIAL USES. USE OF THIS PRODUCT BY A PURCHASER FOR ANY PURPOSE OTHER THAN FOR RESEARCH IS UNAUTHORIZED AND PROHIBITED.

THE IMANIS CELL LINES ARE NOT INTENDED FOR USE IN HUMANS. CELL LINES TRANSFECTED 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: SR

Quality Assurance by: RLV

Effective Date: 05-May-2021