LL/2-Fluc-Neo/eGFP-Puro

Product Description

Product Name: LL/2-Fluc-Neo/eGFP-Puro
Catalog Number: CL073
Lot Number: CL-IM173

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 genes: Firefly luciferase (Fluc)
Enhanced green fluorescent protein (eGFP)
Selection genes: Neomycin (Neo)
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 1) LV-Fluc-P2A-Neo (Imanis #LV011) encoding the firefly luciferase (Fluc) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene (Neo) by a P2A cleavage peptide and 2) LV-eGFP-PGK-Puro (Imanis #LV031) encoding the enhanced green fluorescent protein (eGFP) cDNA under the SFFV promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter. High Fluc and eGFP expressing cells were selected using G418 and 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[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 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.

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.

eGFP and Fluc are immunogenic and may cause tumor rejection in immunocompetent mice. For the most consistent results, immunocompromised mice are recommended for studies.

Additional Considerations

LL/2 cells are loosely adherent. If necessary, culture plates can be coated with poly-D-Lysine prior to use in order to increase cell adherence to the plates.

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 -130°C).

Complete Growth Medium

Dulbecco's Modified Eagle's Medium (DMEM)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
1.25 mg/mL G418 (to maintain high Fluc expression)
2 µg/mL puromycin (to maintain high eGFP expression)

Caution! Typical commercial puromycin stocks are provided at a concentration of 10 mg/mL or 10,000X. G418 and puromycin should NOT be added to the medium until a culture has been well established from the thawed cells (~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.

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 without selection drugs. Centrifuge at ~250 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium without selection drugs. Transfer cells to a T75 flask containing 10 mL complete growth medium without selection drugs.
5. Incubate the culture at 37°C with 5% CO_2. Cells should reach full confluency 3-4 days after thawing.
6. Transfer cells to complete growth medium containing 2 µg/mL puromycin and 1.25 mg/mL G418 after one week.

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 1 mL of 0.25% Trypsin-EDTA solution to the flask and incubate at room temperature until cells have dissociated (~1 min).
4. Neutralize the trypsin by adding 9 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 days. LL/2 cells clump and may begin to detach from flasks prior to reaching 100% confluency. Therefore, cells should be passaged when they reach ~80% confluency. Many cells that become detached remain viable; they can be collected by low-speed centrifugation and re-seeded if necessary.

Freezing Medium

These 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 selection drugs supplemented with 5-10% DMSO.

LL/2-Fluc-Neo/eGFP-Puro

Certificate of Analysis

Testing performed by Imanis Life Sciences

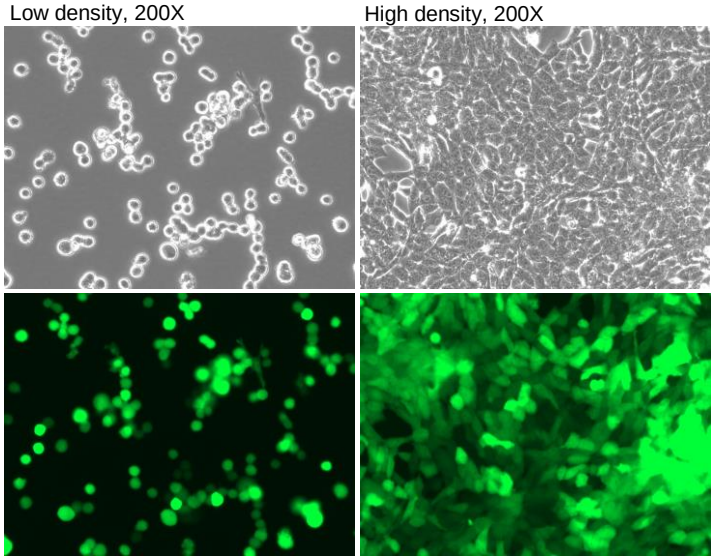
Test description	Result
Post thaw viable cell recovery	98% viability
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
eGFP expression	Pass QC
Average doubling time	10.8 h*

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

Morphology:

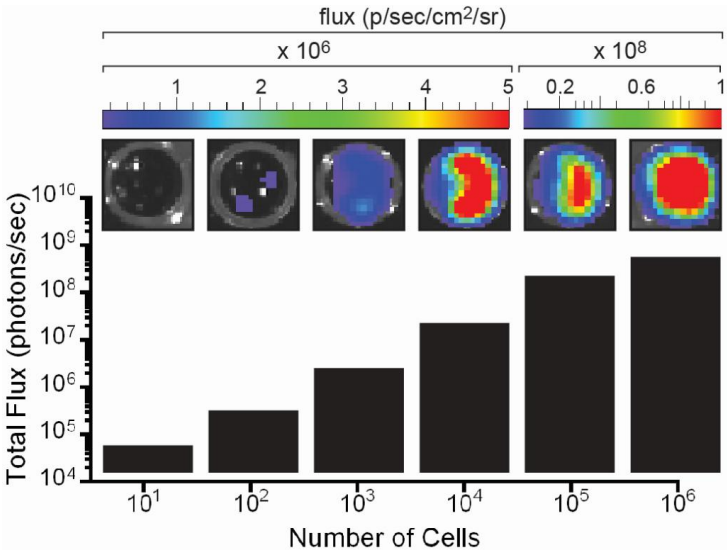
Low density, 200X

High density, 200X



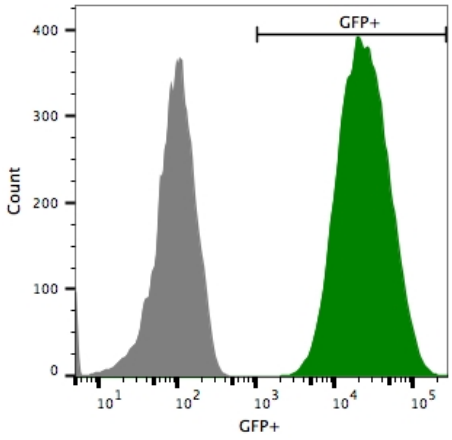
Low and high density photos taken 24 and 72 hours after thawing, respectively.

Luciferase Expression



The indicated number of cells were placed in wells of a 96-well plate. After the addition of 3 mg/mL d-luciferin, the plate was immediately imaged using an IVIS Spectrum. The total flux (photons/sec) was plotted as a function of cell number.

Fluorescence Expression:



LL/2-Fluc-Neo/eGFP-Puro (green) or isotype control (LL/2 parental; 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 LIMTED 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 SALK INSTITUTE ACTIVELY LICENSES ITS PATENTS FOR COMMERCIAL USES, AND A COMMERCIAL USE LICENSE MAY BE AVAILABLE FOR SALK'S WPRE PATENTS. IF YOU WISH TO INQUIRE ABOUT SUCH A LICENSE, PLEASE CONTACT:
Office of Technology Development
The Salk Institute for Biological Studies
10010 North Torrey Pines Road
La Jolla, CA 92037
Phone: (858) 453-4100 extension 1278
Fax: (858) 546-8093

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: JDR
Quality Assurance by: JKM
Effective Date: 04-Oct-2018