

Product Description

Product Name: LL/2-iRFP-Puro
 Catalog Number: CL111
 Lot Number: CL-IM208
 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: Near infrared fluorescent protein (iRFP)
 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 LV-iRFP-P2A-Puro (Imanis #LV032) encoding the near-infrared fluorescent protein (iRFP; ex/em = 690/713) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High iRFP 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 LTR¹.

Mycoplasma Testing

The LL/2-iRFP-Puro cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental LL/2 cell line was authenticated and certified free of interspecies cross contamination by STR profiling with 27 STR loci.

Recommended Uses

LL/2-iRFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation.

Storage Instructions

Remove cells from the dry ice packaging and immediately store in the vapor phase above liquid nitrogen (below -130°C).

References

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

Complete Growth Medium

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

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 pre-warmed complete growth medium without puromycin. Centrifuge cells at ~250 x g for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium without puromycin. Transfer cells to a T75 flask containing 10 mL pre-warmed complete growth medium without puromycin.
5. Incubate the culture at 37°C with 5% CO₂. After 48 hours, replace the culture supernatant with complete growth medium containing 2 µg/mL puromycin. Cells should reach full confluency 2-3 days after thawing.

Subculturing Instructions

Volumes are given for a T75 flask. Increase or decrease as needed. In order to maintain high iRFP expression, it is recommended that cells be subcultured in the presence of 2 µg/mL puromycin. LL/2 cells are loosely adherent, and may begin to detach from flasks prior to reaching 100% confluency. Therefore, cells should be passaged when they reach ~80% confluency.

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 37°C 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% CO₂ incubator.

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

The 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

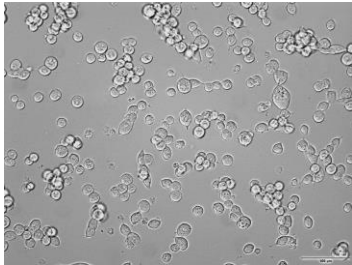
Testing performed by Imanis Life Sciences

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*	10.6 hrs

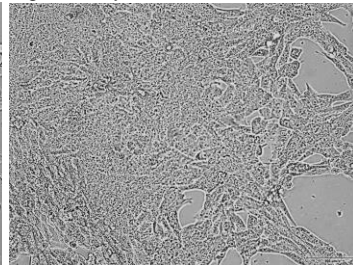
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:

Low density, 200X

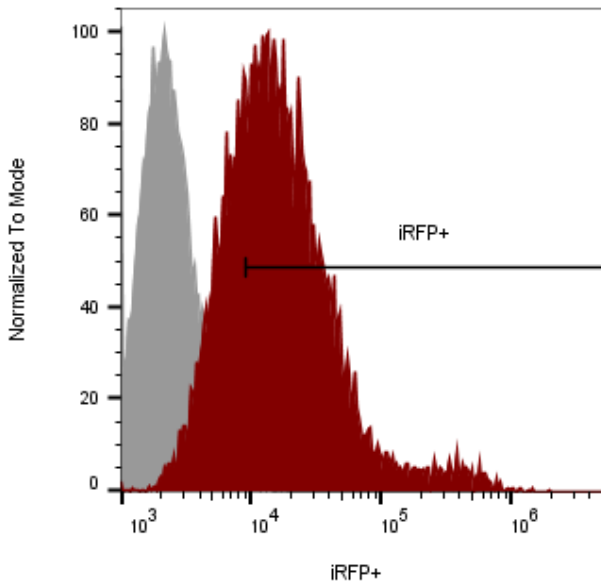


High density, 200X



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

Fluorescence Expression



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

Quality control by: CL

Quality Assurance by: RLV

Effective Date: 05-May-2021

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