LL/2-iRFP-Puro

Product Description

Product Name: LL/2-iRFP-Puro
Catalog Number: CL111
Lot Number: CL-IM109

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 LTR[1].

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Mycoplasma Testing

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

Cell Line Authentication

In light of studies suggesting that 18-36% of cell lines utilized in biomedical research are contaminated or completely misidentified,[2,3] several funding organizations, including NIH, as well as major publishers, including those affiliated with the American Associate for Cancer Research (AACR), require cell lines used in research to be authenticated prior to publication[4,5]. The parental LL/2 cell line used to generate LL/2-iRFP-Puro, 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.

LL/2 cells form tumors post implantation into syngenic C57BL mice[6] and can be used for studying metastasis and cancer chemotherapeutics. The iRFP transgene facilitates noninvasive imaging of implanted cells.

References

[1]Miyoshi et al. J Virol. 1998. 72:8150-8157.
[2]Hughes et al. BioTechniques 2007. 43: 575-586.
[3]Chatterjee et al. Science 2007. 315:928-931.
[4]https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html
[5]http://www.aacrjournals.org/site/InstrAuthors/ifora.xhtml#celllineuse
[6]Bertram et al. Cancer Letters, 1980. 11:63-73.

Storage Instructions

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

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)

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 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% CO_2. After 48 hours, replace the culture supernatant with complete growth medium containing 2 µg/mL puromycin. Cells should reach full confluency 3-4 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 37ºC 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_2 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.

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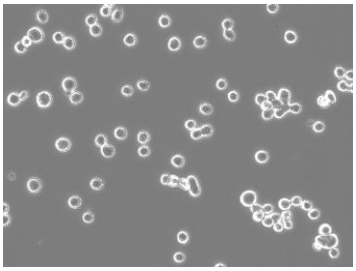
Certificate of Analysis

Testing performed by Imanis Life Sciences

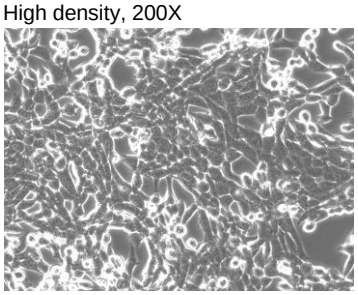
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC

Morphology:

Low density, 200X

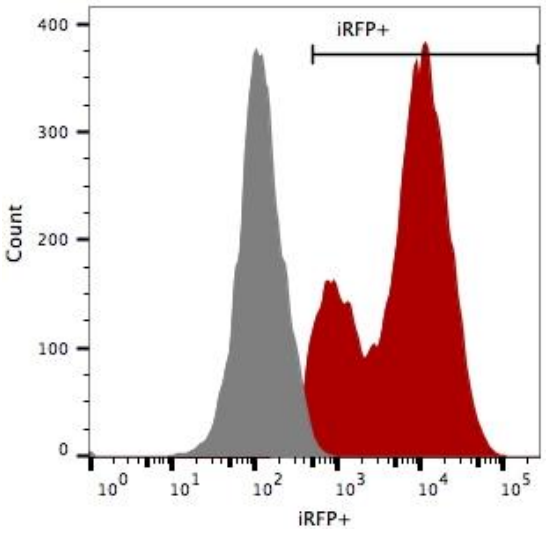


High density, 200X



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.

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