HT1080-Fluc-Neo/eGFP-Puro

Product Description

Product Name: HT1080-Fluc-Neo/eGFP-Puro
Catalog Number: CL104
Lot Number: CL-IM102

Species: Human (*Homo sapiens*)
Tissue: Connective tissue
Cell type: Fibrosarcoma
Parental cells: HT1080 (ATCC® CCL-121™)
Morphology: Epithelial
Growth mode: 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 fibrosarcoma HT1080 cell line (ATCC® CCL-121™). Parental HT1080 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) via 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].

Mycoplasma Testing

The HT1080-Fluc-Neo/eGFP-Puro cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental HT1080 cell line used to generate HT1080-Fluc-Neo/eGFP-Puro was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

Recommended Uses

HT1080-Fluc-Neo/eGFP-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 -130ºC).

References

[1]Miyoshi 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
1.25 mg/mL G418 (to maintain high Fluc expression)
1 µg/mL puromycin (to maintain high eGFP expression)

G418 and 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 G418 and 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 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 selection drugs. Centrifuge cells 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 pre-warmed complete growth medium without selection drugs.
5. Incubate the culture at 37°C with 5% CO_2. After 48 hours, replace the culture supernatant with complete growth medium containing 1.25 mg/mL G418 and 1 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. To maintain high Fluc and eGFP expression, it is recommended that cells be subcultured in the presence of 1.25 mg/mL G418 and 1 µg/mL puromycin. HT1080-Fluc-Neo/eGFP-Puro cells should be passaged when they reach 90-100% 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

HT1080-Fluc-Neo/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 selection drugs supplemented with 5-10% DMSO.

Additional Considerations

HT1080-Fluc-Neo/eGFP-Puro cells can detach from tissue culture surfaces upon repeated washing. Coating culture plates with poly-D-Lysine prior to use can increase cell adherence to the plates during experiments requiring repeated washes or media changes.

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Certificate of Analysis

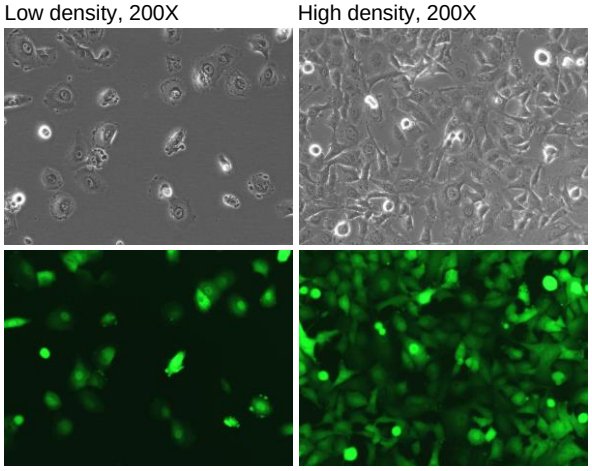
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	92%
Cells per vial	~3 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC
Average doubling time*	30.4 h

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

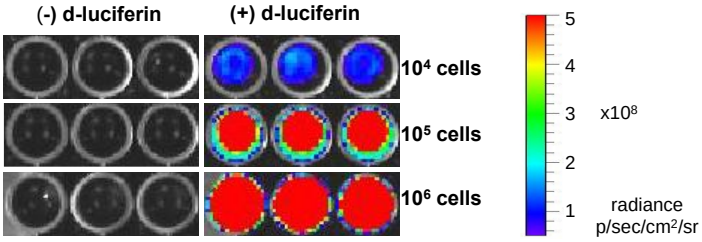
Morphology:

Low density, 200X High density, 200X



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

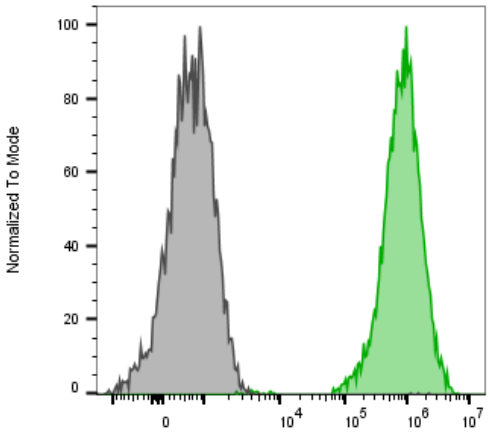
Luciferase Expression



10^4, 10^5, or 10^6 cells were placed in wells of a 96-well plate and 30 µg/mL of d-luciferin was added to the indicated wells. The plate was immediately imaged using a Xenogen IVIS Spectrum.

Quality control by: CDL
Quality Assurance by: RLV
Effective Date: 08-Sep-2021

Fluorescence Expression



HT1080-Fluc-Neo/eGFP-Puro (green) or isotype control (HT1080-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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