

## Product Description

Product Name: HT1080-eGFP-Puro  
 Catalog Number: CL075  
 Lot Number: IMP019

Species: Human (*Homo sapiens*)  
 Tissue: Connective tissue  
 Cell type: Fibrosarcoma  
 Parental cells: HT1080 (ATCC® CCL-121™)\*  
 Morphology: Epithelial  
 Growth mode: Adherent  
 Reporter gene: Enhanced green fluorescent protein (eGFP)  
 Selection gene: Puromycin (Puro)

This is a polyclonal population derived from the fibrosarcoma HT1080 cell line (ATCC® CCL-121™). Parental HT1080 cells were transduced with 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 been tested for mycoplasma contamination and is mycoplasma free.

## Cell Line Authentication

Authentication of the parental HT1080 cell was confirmed by short tandem repeat (STR) profiling.

## Recommended Uses

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.

## Biosafety Notice

This cell line was generated by transduction with a lentiviral vector. Cell lines transduced with lentiviral vectors are classified as biosafety level 2 reagents and should be used under appropriate biosafety level for institutional guidelines.

## 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 µg/mL puromycin

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 (~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. 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 complete growth medium.
5. Incubate the culture at 37°C with 5% CO<sub>2</sub>. Cells should reach full confluency 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.

For maintenance, a subcultivation ratio of 1:10 is recommended. At this ratio cells will be ready for passage approximately every 3 days.

## Freezing Medium

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.

## Certificate of Analysis

Testing performed by Imanis Life Sciences

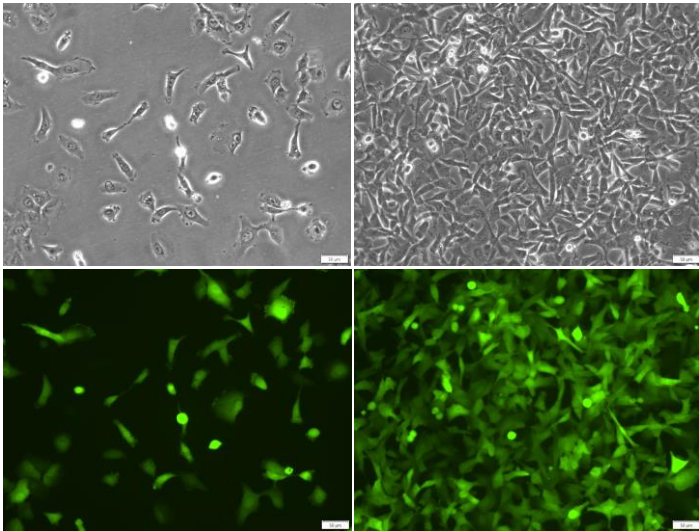
Test description	Result
Post thaw viable cell recovery	98%
Viable cells per vial	~ 3.5 x 10 <sup>6</sup>
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC

Quality control by: AWD  
 Quality Assurance by: RLV  
 Effective Date: 04-May-2023

## Morphology:

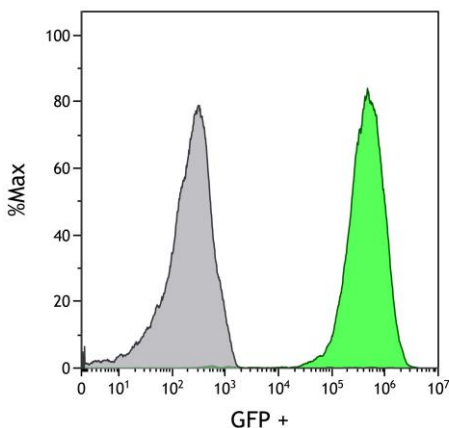
Low density, 200X

High density, 200X



Low- and high- density photos taken after thawing.

## Fluorescence Expression



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

## Legal Disclaimers

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