

## Product Description

Product Name: PC3-eGFP-Neo  
Catalog Number: CL123  
Lot Number: CL-IM190

Species: Human (*Homo sapiens*)  
Cell type: Prostate  
Parental cells: PC3 (ATCC® CRL-1435™)\*  
Morphology: Epithelial  
Growth mode: Adherent  
Reporter gene: Enhanced green fluorescent protein (eGFP)  
Selection gene: Neomycin (Neo)

This is a polyclonal population derived from the prostate PC3 cell line (ATCC® CRL-1435™). Parental PC3 cells were transduced with LV-eGFP-P2A-Neo (Imanis #LV067) encoding enhanced green fluorescent protein (eGFP) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene (Neo) via a P2A cleavage. High eGFP expressing cells were selected using G418. The lentiviral vector is a self-inactivating (SIN) vector 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>.

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## Mycoplasma Testing

This cell line has tested negative for mycoplasma contamination.

## Cell Line Authentication

Authentication of the parental PC3 cell line was confirmed by short tandem repeat (STR) profiling.

## Recommended Uses

These cells are suitable for *in vitro* and *in vivo* experimentation

eGFP is not recommended for whole animal in-live imaging. Rather, samples can be collected post mortem for analysis by conventional fluorescence microscopy.

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.

## 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 Eagles Media (DMEM)  
10% fetal bovine serum (FBS)  
1% Penicillin/Streptomycin  
0.5 mg/mL G418

G418 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 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. 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 2-3 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 1:3 is recommended. At this ratio cells will be ready for passage approximately every 3-4 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 G418 supplemented with 5-10% DMSO.

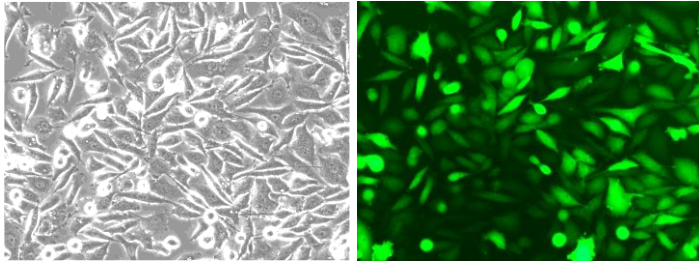
## Certificate of Analysis

Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	99% viability
Sterility	No contamination detected
Mycoplasma	Negative
G418 selection	Pass QC
eGFP expression	Pass QC
Average doubling time	29.3 h*

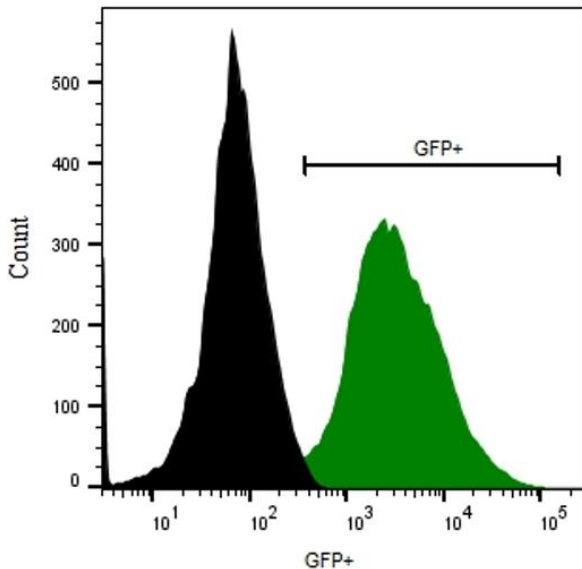
\*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

## Morphology:



Photos taken at 200X, at 72h after thawing.

## Fluorescence Expression:



PC3-eGFP-Neo (green) or isotype control (PC3 Parental; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

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Effective Date: 14-May-2019