HCT116-eGFP-Puro

Product Description

Product Name: HCT116-eGFP-Puro
Catalog Number: CL063
Lot Number: CL-IM172

Species: Human (*Homo sapiens*)
Tissue: Colon
Cell type: Colorectal carcinoma
Parental cells: HCT116 (ATCC® CCL-247™)*
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Enhanced green fluorescent protein (eGFP)
Selection gene: Puromycin (Puro)

This is a polyclonal population derived from the colorectal carcinoma HCT116 cell line (ATCC® CCL-247™). Parental HCT116 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 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[1].

*The ATCC trademark and any and all ATCC catalog numbers are trademarks of the American Type Culture Collection.

Mycoplasma Testing

This cell line has tested negative for mycoplasma contamination.

Cell Line Authentication

Studies suggest that 18-36% of cell lines utilized in biomedical research are contaminated or completely misidentified,[2,3] and several funding organizations and major publishers require cell lines to be authenticated prior to publication[4,5]. Authentication of the parental HCT116 cell line was confirmed by short tandem repeat (STR) profiling with 9 STR loci.

Recommended Uses

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

HCT116 cells are a xenograph model for colorectal carcinoma and will form primary tumors and distant metastases (lung, liver, and lymph nodes) post-implantation into immunosupressed mice[6]. eGFP is not recommended for whole animal in-live imaging. Rather, samples can be collected postmortem for analysis by conventional fluorescence microscopy or flow cytometry.

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]Yang et al. Anticancer Res. 1997. 17:3463-3468.

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 Modifies Eagles Media (DMEM)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
1 µg/mL puromycin (to maintain high eGFP expression)

(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 (~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 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 complete growth medium without puromycin.
5. Incubate the culture at 37°C with 5% CO_2. Cells should reach full confluency 2-3 days after thawing.
6. Transfer cells to complete growth medium containing 1 µg/mL puromycin after 1 week.

Subculturing Instructions

Volumes are given for a T75 flask; increase or decrease as needed. To maintain high eGFP expression, it is recommended that cells be subcultured in the presence of 1 µg/mL puromycin.

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

These cells can be amplified and used to generate additional frozen stocks. Cryopreservation of low passage stocks is recommended. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium without puromycin supplemented with 5-10% DMSO.

Additional Considerations

HCT116 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.

HCT116-eGFP-Puro

Certificate of Analysis

Testing performed by Imanis Life Sciences

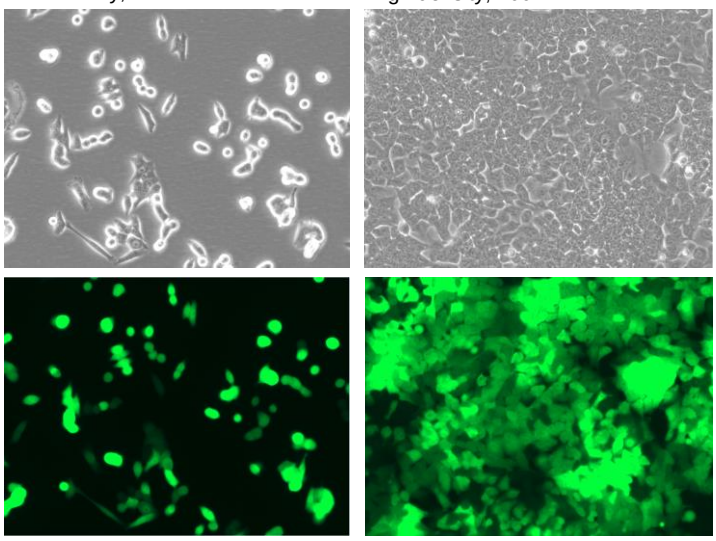
Test description	Result
Post thaw viable cell recovery	97% viability
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
eGFP expression	Pass QC
Average doubling time	15.8 h*

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

Morphology:

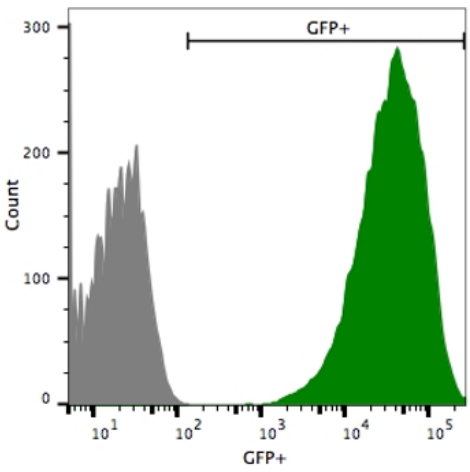
Low density, 200X

High density, 200X



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

Fluorescence Expression:



HCT116-eGFP-Puro (green) or isotype control (HCT116 parental; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

Quality control by: JDR
Quality Assurance by: RLV
Effective Date: 06-Sep-2018

Legal Disclaimers

LIMITED PRODUCT WARRANTY
THIS WARRANTY LIMITS OUR LIABILITY TO REPLACEMENT OF THIS PRODUCT. NO OTHER WARRANTIES OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, ARE PROVIDED BY IMANIS. IMANIS SHALL HAVE NO LIABILITY FOR ANY DIRECT, INDIRECT, CONSEQUENTIAL, OR INCIDENTAL DAMAGES ARISING OUT OF THE USE, THE RESULTS OF USE, OR THE INABILITY TO USE THIS PRODUCT.

FOR *IN VITRO* USE ONLY. THIS CERTIFICATE IS A DECLARATION OF ANALYSIS AT THE TIME OF MANUFACTURE.

PURCHASER NOTIFICATION

LIMITED LICENSE NOTICE – RESEARCH USE ONLY

IMANIS LIFE SCIENCES HAS A LIMTED LICENSE UNDER PATENTS OWNED BY THE SALK INSTITUTE FOR BIOLOGICAL STUDIES THAT PERMITS IMANIS LIFE SCIENCES TO SELL PRODUCTS CONTAINING WPRE FOR RESEARCH USE ONLY AND NOT FOR ANY COMMERCIAL USES. EXCLUDED COMMERCIAL USES INCLUDE WITHOUT LIMITATION MANUFACTURING, PROVIDING A SERVICE, THERAPEUTIC, DIAGNOSTIC AND PROPHYLACTIC USES, AND ANY OTHER COMMERCIAL USES. USE OF THIS PRODUCT BY A PURCHASER FOR ANY PURPOSE OTHER THAN FOR RESEARCH IS UNAUTHORIZED AND PROHIBITED.

THE SALK INSTITUTE ACTIVELY LICENSES ITS PATENTS FOR COMMERCIAL USES, AND A COMMERCIAL USE LICENSE MAY BE AVAILABLE FOR SALK'S WPRE PATENTS. IF YOU WISH TO INQUIRE ABOUT SUCH A LICENSE, PLEASE CONTACT:
Office of Technology Development
The Salk Institute for Biological Studies
10010 North Torrey Pines Road
La Jolla, CA 92037
Phone: (858) 453-4100 extension 1278
Fax: (858) 546-8093

THE IMANIS CELL LINES ARE NOT INTENDED FOR USE IN HUMANS. CELL LINES TRANSDUCED WITH LENTIVIRAL VECTORS ARE CLASSIFIED AS BIOSAFETY LEVEL 2 REAGENTS AND SHOULD BE USED UNDER THE APPROPRIATE BIOSAFETY LEVEL PER INSTITUTIONAL GUIDELINES.
THE PURCHASER AGREES THAT IMANIS MATERIALS DESIGNATED AS BIO-SAFETY LEVEL 2 CONSTITUTE KNOWN PATHOGENS AND THAT OTHER IMANIS MATERIALS NOT SO DESIGNATED AND ANY PROGENY OR MODIFICATION MAY BE PATHOGENIC UNDER CERTAIN CONDITIONS. PURCHASER ASSUMES ALL RISK AND RESPONSIBILITY IN CONNECTION WITH THE RECEIPT, HANDLING, STORAGE, DISPOSAL, TRANSFER AND USE OF THE IMANIS MATERIALS INCLUDING WITHOUT LIMITATION TAKING ALL APPROPRIATE SAFETY AND HANDLING PRECAUTIONS TO MINIMIZE HEALTH OR ENVIRONMENTAL RISK. PURCHASER AGREES THAT ANY ACTIVITY UNDERTAKEN WITH THE IMANIS MATERIALS AND ANY PROGENY OR MODIFICATION WILL BE CONDUCTED IN COMPLIANCE WITH ALL APPLICABLE GUIDELINES, LAWS AND REGULATIONS. THE IMANIS MATERIAL, ANY OTHER IMANIS PRODUCTS, AND ANY TECHNICAL INFORMATION AND ASSISTANCE PROVIDED BY IMANIS ARE PROVIDED "AS IS", WITHOUT WARRANTIES OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, MANUFACTURE ACCORDING TO cGMP STANDARDS, TYPICALITY, SAFETY, ACCURACY AND NON-INFRINGEMENT. IN NO EVENT SHALL IMANIS, ITS PARENTS, SUBSIDIARIES, DIRECTORS, OFFICERS, AGENTS, EMPLOYEES, ASSIGNS, SUCCESSORS AND AFFILIATE (COLLECTIVELY "IMANIS INDEMNIFIED PARTIES") BE LIABLE FOR INDIRECT, SPECIAL, INCIDENTAL OR CONSEQUENTIAL DAMAGES OF ANY KIND IN CONNECTION WITH OR ARISING OUT OF THIS AGREEMENT (WHETHER IN CONTRACT, TORT, NEGLIGENCE, STRICT LIABILITY, STATUTE OR OTHERWISE) EVEN IF IMANIS HAS BEEN ADVISED, KNEW OR SHOULD HAVE KNOWN OF THE POSSIBILITY OF SUCH DAMAGES, INCLUDING, BUT NOT LIMITED TO, LOST PROFITS, COST OF CAPITAL, COST OF SUBSTITUTE PRODUCTS OR CLAIMS OF LICENSEE'S CUSTOMERS FOR SUCH DAMAGE. IN NO EVENT SHALL IMANIS' CUMULATIVE LIABILITY EXCEED THE ACTUAL AMOUNTS PAID BY PURCHASER UNDER THIS AGREEMENT FOR THE TWELVE (12) MONTH PERIOD PRECEDING THE DATE OF THE EVENT GIVING RISE TO THE CLAIM. THE PROVISIONS OF THIS SECTION SHALL SURVIVE THE EXPIRATION OR TERMINATION OF THIS AGREEMENT AND SHALL APPLY EVEN IF THE LIMITED REMEDY SPECIFIED IN THIS AGREEMENT IS FOUND TO HAVE FAILED OF ITS ESSENTIAL PURPOSE.