

Product Description

Product Name: HCT116-Fluc-Neo/eGFP-Puro
 Catalog Number: CL070
 Lot Number: CL-IM65

Shipping conditions: Dry ice
 Storage conditions: Store in vapor phase above liquid nitrogen

Species: Human (*Homo sapiens*)
 Cell type: Colorectal carcinoma
 Morphology: Epithelial
 Growth mode: Adherent
 Reporter genes: Firefly luciferase (Fluc)
 Enhanced green fluorescent protein (eGFP)
 Selection genes: Neomycin (Neo)
 Puromycin (Puro)
 Media: DMEM, 10% FBS, 1% Penicillin/Streptomycin,
 1 µg/mL puromycin, 0.5 mg/mL G418
 Subculture: split confluent culture 1:10 every 4-5 days using
 0.25% trypsin/EDTA
 Incubation: 37°C with 5% CO₂

Description: HCT116-Fluc-Neo/eGFP-Puro is a polyclonal population of the human colorectal carcinoma HCT116 cell line transduced with lentiviral vectors encoding 1) 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 (Imanis #LV012) and 2) the enhanced green fluorescent protein (eGFP) cDNA under the SFFV promoter and the puromycin resistance gene (Puro) under the phosphoglycerate kinase (PGK) promoter (Imanis #LV031). 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 (Miyoshi et al., J Virol. 1998).

Cell line Authentication: Authentication of the parental HCT116 cell line was performed by Short tandem repeat (STR) profiling with 9 STR loci including CSF1PO, D13S317, D16S539, D5S818, D7S820, TH01, TPOX, vWA and sex chromosome marker *Amelogenin*. STR profiling of HCT116 cells are verified and there is no interspecies cross contamination detected.

It has been estimated that ~18-36% of cell lines utilized in biomedical research are contaminated or completely misidentified (Hughes et al., BioTechniques 2007). Consequently, verification of cell line identity is of critical significance. Several funding organizations, including NIH, and major publishers, such as those affiliated with the American Association for Cancer Research (AACR), have established requirements for cell line authentication prior to publication. More information can be found in the links below.

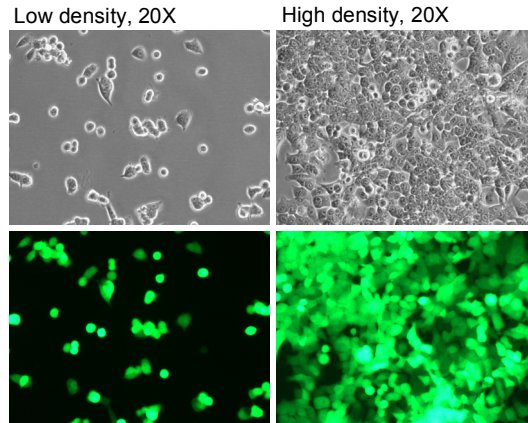
<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-08-017.html>
<http://www.aacrjournals.org/site/InstrAuthors/fora.xhtml#celllineuse>

Certificate of Analysis

Testing performed by Imanis Life Sciences:

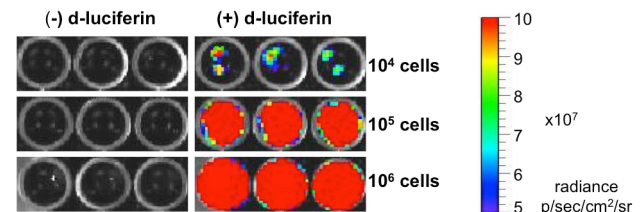
Test description	Result
Post thaw viable cell recovery	Pass QC
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Puromycin selection	Pass QC
Luciferase expression	Pass QC
Fluorescence expression	Pass QC

Morphology:



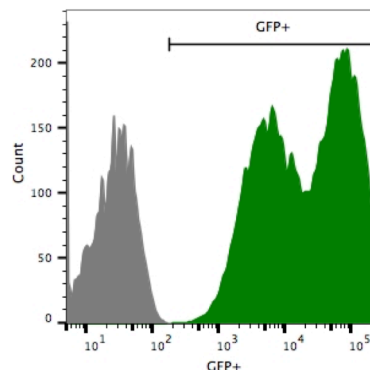
Low and high density photos taken 24 and 66 hours after thawing, respectively.

Luciferase Expression



10⁴, 10⁵, or 10⁶ cells were placed in wells of a 96-well plate and 0.3 mg of d-luciferin was added to the indicated wells. The plate was immediately imaged using a Xenogen IVIS Spectrum.

Fluorescence Expression:



HCT116-Fluc-Neo/eGFP-Puro (green) or isotype control (HCT116-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry (20,000 events).

Quality Control by: RLV

Quality Assurance by: SPR

Effective Date: 11/24/15

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.

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