

Product Description

Product Name: HCT116-iRFP-Puro
Catalog Number: CL074
Lot Number: CL-IM71

Species: Human (*Homo sapiens*)
Tissue: Colon
Cell type: Colorectal carcinoma
Parental cells: HCT116 (ATCC® CCL-247™) *
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Near-infrared fluorescent protein (iRFP)
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-iRFP-P2A-Puro (Imanis #LV032) encoding the near-infrared fluorescent protein (iRFP; ex/em = 690/713) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the puromycin resistance gene (Puro) via a P2A cleavage peptide. High iRFP 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 LTR¹.

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

Mycoplasma Testing

The HCT116-iRFP-Puro cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental HCT116 cell line was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

Recommended Uses

HCT116-iRFP-Puro cells are suitable for *in vitro* and *in vivo* experimentation.

References

¹Miyoshi 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 Eagle's Medium (DMEM)
10% fetal bovine serum (FBS)
1% Penicillin/Streptomycin
1 µg/mL puromycin (to maintain high iRFP 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 37°C 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 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 pre-warmed complete growth medium without puromycin.
5. Incubate the culture at 37°C with 5% CO₂. After 48 hours, replace the culture supernatant with complete growth medium containing 1 µg/mL puromycin. Cells should reach full confluency 2-3 days after thawing.

Subculturing Instructions

Volumes are given for a T75 flask; increase or decrease as needed. To maintain high iRFP expression, it is recommended that cells be subcultured in the presence of 1 µg/mL puromycin. The 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 37°C 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% CO₂ 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

The 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.

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.

Certificate of Analysis

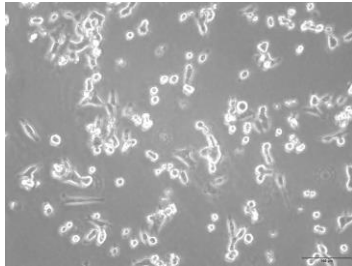
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	94%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin selection	Pass QC
Fluorescence expression	Pass QC
Average Doubling Time*	16.3 hrs

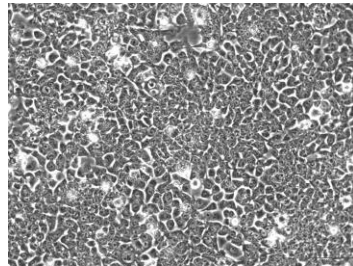
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology:

Low density, 200X

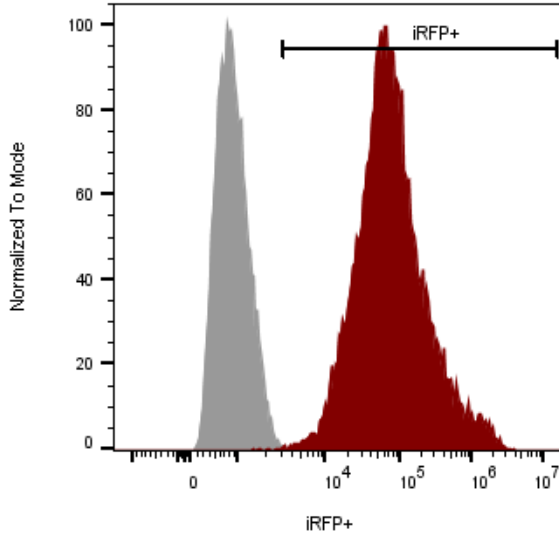


High density, 200X



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

Fluorescence Expression



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

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Quality control by: CL

Quality Assurance by: RLV

Effective Date: 05-May-2021