

Product Description

Product Name: A375-iRFP-Neo
Catalog Number: CL088
Lot Number: CL-IM85

Species: Human (*Homo sapiens*)
Tissue: Skin
Cell type: Malignant melanoma
Parental cells: A375 (ATCC® CRL-1619™)
Morphology: Epithelial
Growth mode: Adherent
Reporter gene: Near infrared fluorescent protein (iRFP)
Selection gene: Neomycin (Neo)

This is a polyclonal population derived from the malignant melanoma A375 cell line (ATCC® CRL-1619™). Parental A375 cells were transduced with LV-iRFP-P2A-Neo (Imanis #LV033) encoding the near infrared fluorescent protein (iRFP; ex/em = 690/713 nm) cDNA under the spleen focus-forming virus (SFFV) promoter linked to the neomycin resistance gene (Neo) via a P2A cleavage peptide. High iRFP expressing cells were selected using G418. 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.

Mycoplasma Testing

The A375-iRFP-Neo cell line has been tested for mycoplasma contamination and is certified mycoplasma free.

Cell Line Authentication

The parental A375 cell line was authenticated and certified free of interspecies cross contamination by STR profiling with 9 STR loci.

Recommended Uses

A375-iRFP-Neo cells are suitable for *in vitro* and *in vivo* experimentation.

The iRFP transgene in the A375-iRFP-Neo cells facilitates *in vivo* noninvasive imaging of implanted cells.

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
0.6 mg/mL G418 (to maintain high iRFP expression)

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 (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 G418. Centrifuge cells at ~250 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium without G418. Transfer cells to a T75 flask containing 10 mL pre-warmed complete growth medium without G418.
5. Incubate the culture at 37°C with 5% CO₂. After 48 hours, replace the culture supernatant with complete growth medium containing 0.6 mg/mL G418. Cells should reach full confluency 3-4 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 0.6 mg/mL G418. A375-iRFP-Neo 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

A375-iRFP-Neo 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.

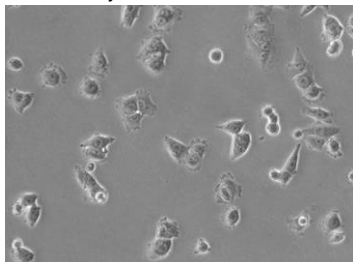
Certificate of Analysis

Testing performed by Imanis Life Sciences

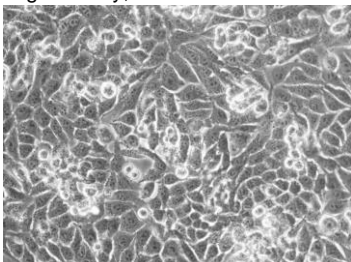
Test description	Result
Post thaw viability	97%
Sterility	No contamination detected
Mycoplasma	No contamination detected
Neomycin selection	Pass QC
Fluorescence expression	Pass QC

Morphology:

Low density, 20X

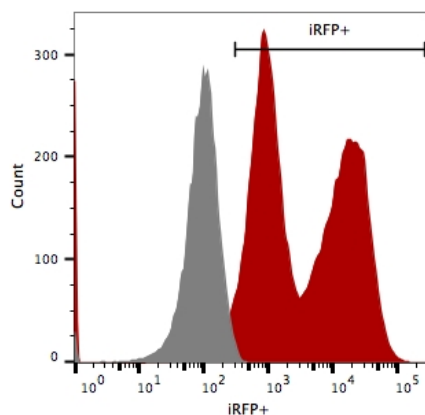


High density, 20X



Low and high density photos taken 25 and 70 hours after thawing, respectively.

Fluorescence Expression



A375-iRFP-Neo (red) or isotype control (A375-Fluc-Puro; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry (20,000 events).

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Quality control by: AMW
Quality Assurance by: RLV
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