K562-Fluc-EmGFP

Product Description

Product Name: K562-Fluc-EmGFP
Catalog Number: CL169
Lot Number: IMP012

Species: Human (*Homo sapiens*)
Tissues: Bone marrow
Cell type: Chronic myelogenous leukemia
Parental cells: K562 (ATCC® CCL-243™)*
Morphology: Lymphoblast
Growth mode: Suspension
Reporter genes: Firefly luciferase (Fluc)
Emerald green fluorescent protein (EmGFP)

This is a cell line derived from the human chronic myelogenous leukemia K562 cell line (ATCC® CCL-243™). Parental K562 cells were transduced with LV-Luc2-P2A-EmGFP (Imanis #LV050) encoding the firefly luciferase (Luc2; Fluc) cDNA under the spleen focus-forming virus (SFFV) promoter and linked to the Emerald green fluorescent protein (EmGFP) cDNA via a P2A cleavage peptide. Luciferase and EmGFP-expressing cells were selected using a methylcellulose-based semi-solid medium. 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 been tested for mycoplasma contamination and is mycoplasma free.

Recommended Uses

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

The luciferase transgene facilitates non-invasive *in vivo* bioluminescence imaging. EmGFP is not recommended for in-life imaging but can be used for post-mortem analyses.

Cell Line Authentication

The parental K562 cell line was purchased directly from ATCC™. ATCC™ authenticated the K562 parental cells by STR profiling.

References

[1]Miyoshi et al. J Virol. 1998. 72:8150-8157.

Biosafety Notice

This cell line was generated by transduction with a lentiviral vector. Cell lines transduced with lentiviral vectors are classified as biosafety level 2 reagents and should be used under appropriate biosafety level for institutional guidelines.

Storage Instructions

Remove cells from the dry ice packaging and immediately store in the vapor phase above liquid nitrogen (below -130ºC).

Complete Growth Medium

Iscove's Modified Dulbecco's Medium (IMDM)
15% fetal bovine serum (FBS)
1% Penicillin/Streptomycin

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. Centrifuge cells at ~200 x *g* for 3-5 min.
4. Remove supernatant and resuspend cells in 1 mL complete growth medium. Remove an aliquot for counting.
5. Dilute the cells further with growth medium to achieve a final density between 1 and 2 x 10^6 cells/mL. Transfer the cells to a T25 or T75 flask based on volume.
6. Incubate the culture at 37°C with 5% CO_2.

Subculturing Instructions

The cells should be subcultured as needed to maintain a density between 5 x 10^5 and 2 x 10^6 cells/mL. The cells can be passaged by dilution in fresh complete growth medium. Regular passage using centrifugation as described below is recommended to limit the amount of debris in cultures.

1. Pipet the cell suspension gently to dislodge any cells loosely attached to the culture flask. Transfer the desired volume of the cells to a conical tube.
2. Centrifuge at ~150 x g for 3 min. (Note: a short, low speed spin is recommended to limit the amount of cell debris in the pellet.)
3. Remove supernatant and resuspend cells in complete growth medium. Transfer to an appropriate sized flask.

Freezing Medium

These cells can be amplified and used to generate additional frozen stocks. Preparation of low passage frozen stocks is highly recommended. Frozen stocks should be preserved in a designated cryopreservation medium or in complete growth medium supplemented with 5-10% DMSO.

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Certificate of Analysis

Testing performed by Imanis Life Sciences

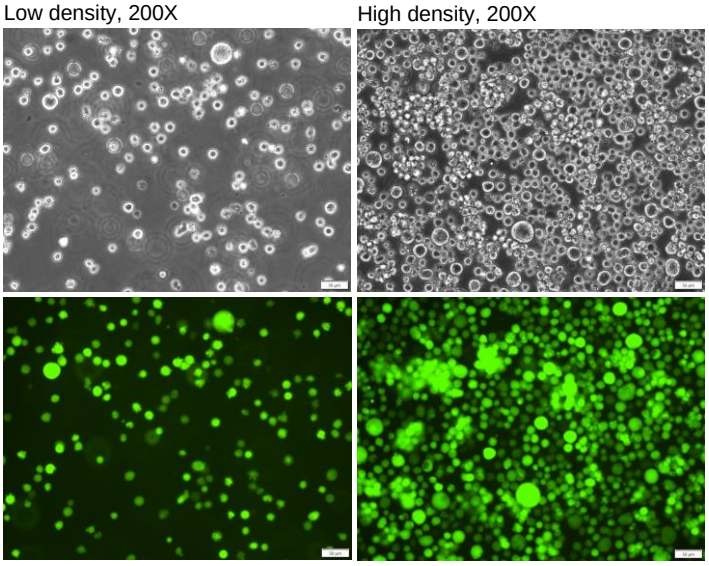
Test description	Result
Post thaw viable cell recovery	91%
Cells per vial	~ 7 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Luciferase expression	Pass QC
Fluorescence expression	Pass QC
Average doubling time	35.9 hours*

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

Morphology

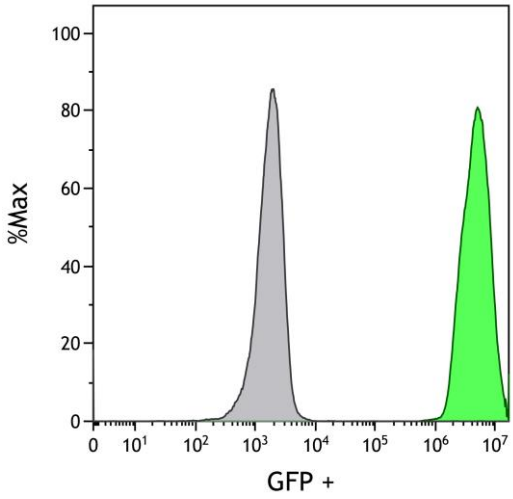
Low density, 200X

High density, 200X



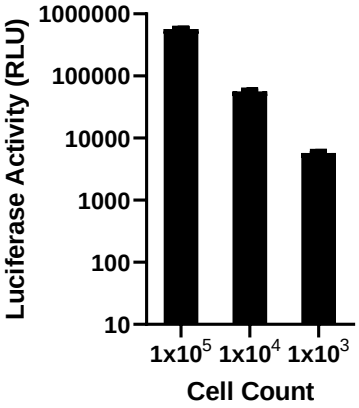
Low- and high-density photos taken at various times after thawing.

GFP Expression



K562-Fluc-EmGFP (green) or isotype control (K562 Parental; grey) cells were fixed with paraformaldehyde and analyzed by flow cytometry.

Luciferase Expression:



The indicated number of cells were placed in wells of a 96-well plate. After the addition of 15 mg/mL d-luciferin, bioluminescence was immediately read using a microplate reader.

Quality control by: AWD
Quality Assurance by: RLV
Effective Date: 10-Jan-2023

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