K562-Fluc-Puro/HuBCMA-Neo

Product Description

Product Name: K562-Fluc-Puro/HuBCMA-Neo
Catalog Number: CL192
Lot Number: IMP049

Species: Human (*Homo sapiens*)
Tissues: Bone marrow
Cell type: Chronic myelogenous leukemia
Parental cells: K562 (ATCC® CCL-243™)*
Morphology: Lymphoblast
Growth mode: Suspension
Reporter gene: Firefly luciferase (Fluc)
Transgene: Human B Cell Maturation Antigen (BCMA)
Selection genes: Puromycin (Puro)
Neomycin (Neo)

This is a cell line derived from the human chronic myelogenous leukemia K562 cell line (ATCC® CCL-243™). Imanis K562-Fluc-Puro cells (Imanis #CL171) were transduced with a lentiviral encoding the Human B cell maturation antigen (BCMA) cDNA under the spleen focus-forming virus (SFFV) promoter and the neomycin resistance gene (Neo) under the phosphoglycerate kinase (PGK) promoter. High Fluc- and BCMA expressing populations were selected using G418 followed by selection using a semi-solid methyl-cellulose-based medium. 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[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.

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 -130°C).

Complete Growth Medium

Iscove's Modified Dulbecco's Medium (IMDM)
15% Fetal Bovine Serum (FBS)
1% Penicillin/Streptomycin
6 µg/mL Puromycin
1 mg/mL G418

Puromycin and 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 puromycin and G418 to the growth medium.

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 (~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 ~300 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 of 1 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 appropriately 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 without selection antibiotics supplemented with 5-10% DMSO.

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Certificate of Analysis

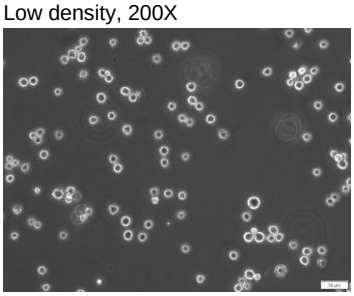
Testing performed by Imanis Life Sciences

Test description	Result
Post thaw viable cell recovery	88%
Cells per vial	~ 7 x 10^6
Sterility	No contamination detected
Mycoplasma	No contamination detected
Puromycin expression	Pass QC
Luciferase expression	Pass QC
Neomycin expression	Pass QC
Human BCMA expression	Pass QC
Average doubling time	20.3 hours*

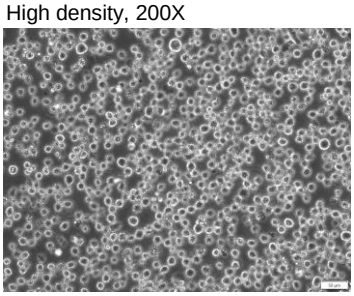
*Doubling time represents the average doubling time during logarithmic growth. This value should be used for general estimation only.

Morphology

Low density, 200X

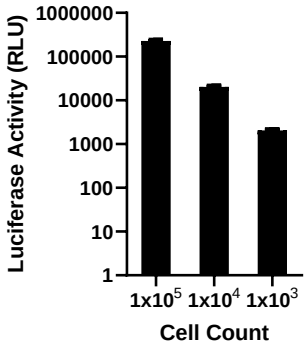


High density, 200X



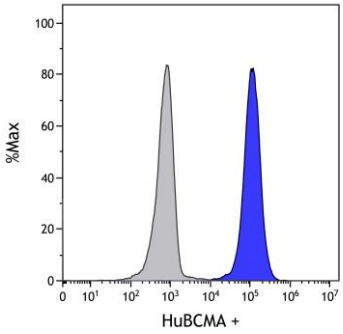
Low- and high-density photos taken at various times after thawing.

Luciferase Expression:



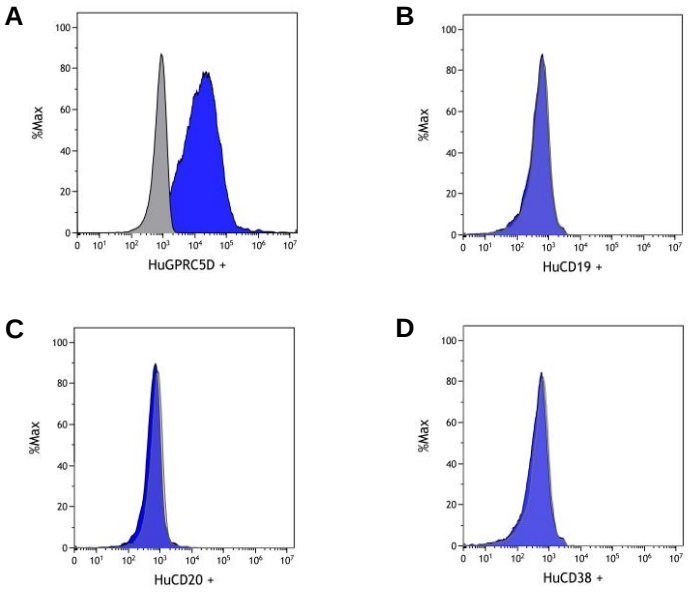
The indicated number of cells were placed in wells of a 96-well plate. After the addition of 15 mg/mL d-luciferin, bioluminescence was immediately read using a microplate reader.

BCMA Expression:



K562-Fluc-Puro/HuBCMA-Neo cells were stained with an anti-HuBCMA (blue) or isotype control (grey) antibody and analyzed by flow cytometry.

Expression Profiling of Surface Markers



K562-Fluc-Puro/HuBCMA-Neo (blue) cells were stained with an anti-HuGPRC5D antibody (A), anti-HuCD19 antibody (B), anti-HuCD20 antibody (C), anti-HuCD38 antibody (D), or isotype control antibody (grey; all panels) and analyzed by flow cytometry.

Quality control by: AWD
Quality Assurance by: RLV
Effective Date: 18-Oct-2024

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