BLaER1 Human B-cell Precursor Leukemia Cell Line

Cancer Cell Line

Cat. # SCC165

Pack size: ≥1x10^6 viable cells/vial

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THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Store in liquid nitrogen

Data Sheet

Background

Induction of differentiation is a therapeutic approach for cancer. Treatment of leukemias with differentiation-inducing factors in combination with pro-apoptotic compounds has been shown to be highly effective.[1] Ectopic expression of the transcription factor C/EBPα causes reprogramming of leukemia cells, resulting in expression of a normal cell phenotype.

The BLaER1 cell line is a tamoxifen-inducible derivative of the RCH-ACV B-cell leukemia cell line genetically modified using a retrovirus vector to express C/EBPα fused with the estrogen receptor hormone binding domain (ER) and GFP.[2,3] A study demonstrated that BLaER1 cells could efficiently convert into cells exhibiting increased adherent, phagocytic and quiescent properties with a transcriptome resembling normal macrophages.[2] Cells retained their phenotype even when C/EBPα was inactivated, a characteristic of genuine cell reprogramming. C/EBPα induction also impaired cell tumorigenicity after transplantation into immunodeficient mice. BLaER1 is thus a model for highly efficient transdifferentiation of B-cell-derived human cancer cells.

Source

BLaER1 cells are a single subclone isolated from C/EBPαER-GFP-transfected RCH-ACV acute lymphoblastic leukemia (ALL) cells sorted for GFP expression. The parental cell line was obtained from the bone marrow of a female with chromosome translocation 1;19 and trisomy 8.[4]

Short tandem repeat (STR) Profile

D3S1358: 15	D16S539: 11
TH01: 6, 8	CSF1PO: 10, 11
D21S11: 29, 31.2	Penta D: 8.4, 10
D18S51: 13,16	vWA: 16, 17
Penta E: 7, 11	D8S1179: 13, 15
D5S818: 11, 12	TPOX: 8, 11
D13S317: 9, 14	FGA: 23, 26
D7S820: 10, 11	Amelogenin: X

Cancer cell lines are inherently genetically unstable. Genetic instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from mouse, rat, chinese hamster, Golden Syrian hamster, and non-human primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services
- Cells are negative for mycoplasma contamination.

Storage and Handling

BLaER1 cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

References

1. Waxman, S. (2000). Differentiation therapy in acute myelogenous leukemia (non-APL). *Leukemia* 14(3):491-496.
2. Rapino F *et al.* (2013) C/EBPα induces highly efficient macrophage transdifferentiation of B lymphoma and leukemia cell lines and impairs their tumorigenicity. *Cell Reports* 3(4): 1153-1163.
3. Rapino F *et al.* (2017) Erratum for C/EBPα induces highly efficient macrophage transdifferentiation of B lymphoma and leukemia cell lines and impairs their tumorigenicity. *Cell Reports* 19(6): 1281.
4. Jack I *et al.* (1986) RCH-ACV: a lymphoblastic leukemia cell line with chromosome translocation 1;19 and trisomy 8. *Cancer Genet. Cytogenet.* 19(3-4):261-269.

Rev.1.0/2018-08-24/SCC165/AN

Protocols

BLaER1 are suspension cells that are very small in size and proliferate rapidly.

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   BLaER1 Expansion Medium: Cells are thawed and expanded in RPMI-1640 with 25 mM HEPES (Sigma Cat. No. R5886-500ML) supplemented with 1X L-Glutamine (Cat. No. TMS-002-C), 10% heat-inactivated FBS (Cat. No. ES-009-B), and 1X β-mercaptoethanol (Cat. No. ES-007-E).
2. Remove the vial of frozen BLaER1 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   IMPORTANT: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of BLaER1 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   IMPORTANT: Do not vortex the cells.
7. Centrifuge the tube at 300 x g for 2-3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 15-20 mL of BLaER1 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37°C in a humidified incubator with 5% CO_2.

Subculturing Cells

BLaER1 suspension cells require media replenishment every 2-3 days. Passage cells when the cell density is at 2 – 2.5 million cells/mL.

1. Remove flask from incubator, tighten cap and place in tissue culture hood.
2. Dislodge any cells that may adhere to the flask by firmly rapping the side of the flask with the palm of the hand and gently swirl the medium over the cells to mix. Visually inspect flask to ensure the cells have been dislodged and the suspension is free of contaminants.
3. Determine cell count and viability using a hemocytometer or automated cell counter.
4. Cells are typically plated at a density of 250,000 cells/mL.

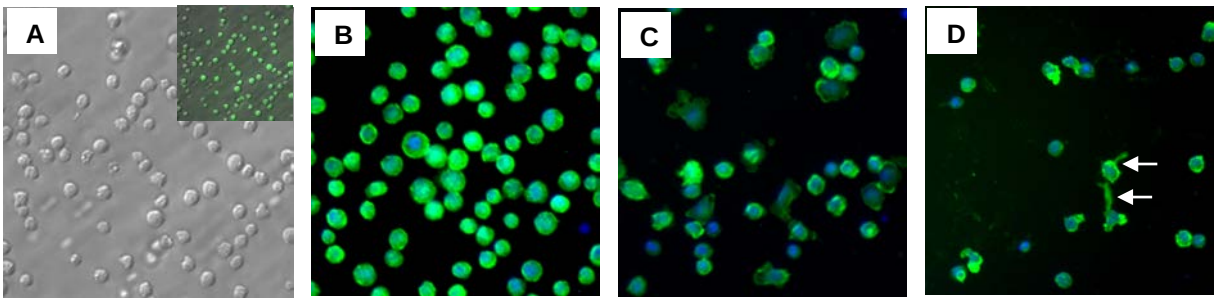
Transdifferentiation

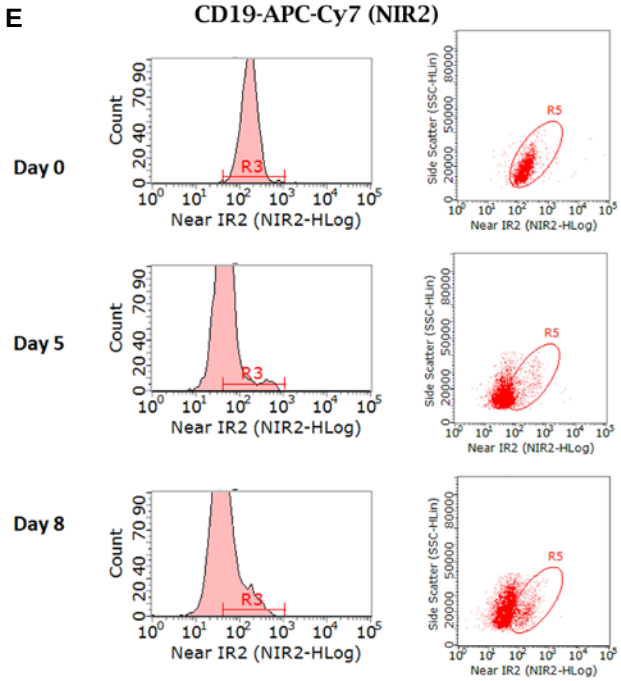
1. To induce transdifferentiation, set a cell concentration of 500,000 cells/mL.
2. C/EBPα is induced by addition of 100 nM 17-beta estradiol (E2) (Cat. No. E8875) and grown with 10 ng/mL of human IL-3 (Cat. No. GF335) and 10 ng/mL human M-CSF (Cat. No. H6916-10UG).
3. There is no need to change the medium or add more cytokines during the process. It takes 7-8 days to transdifferentiate.

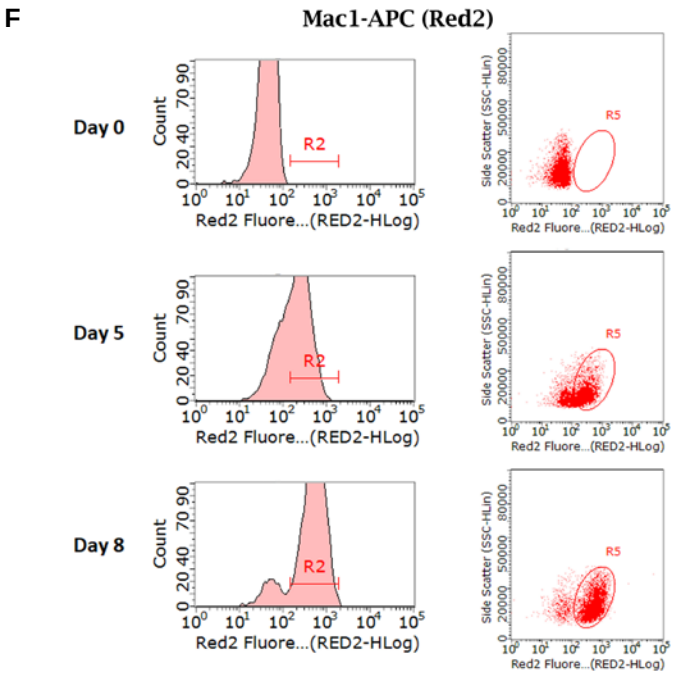
Cryopreservation of Cells

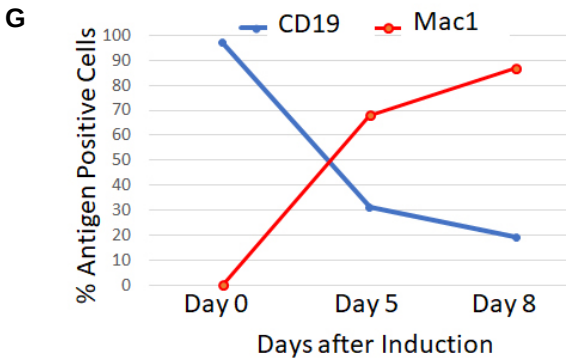
BLaER1 cells may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

Representative Data









Day	CD19	Mac1
0	97 %	0 %
5	31 %	68 %
8	19 %	87 %

Figure 1. BLaER1 cells are small suspension cells (**A**) that are GFP positive (**A, inset**). Uninduced cells stained with Phalloidin-FITC to detect F-actin (**B**). Induced cells at days 5 (**C**) and 8 (**D**) stained with Phalloidin-FITC. Induced cells acquired an irregular morphology and developed F-actin-positive lamellipodia (**D, arrow**). Nuclei were labeled with Dapi.

Undifferentiated cells express high levels of CD19 marker (**E, G**) whereas differentiated cells express high levels of the macrophage marker Mac1 (**F, G**). BLaER1 cells were differentiated for 8 days with 100 nM 17-beta estradiol (E2), 10 ng/mL of hIL3 and 10 ng/mL hr-CSF-1. The morphological change to macrophage-like cells after trans-differentiation is observed via F-actin staining (**C, D**).

Kinetics of trans-differentiation of CD19 and Mac1 expression (**G**) as determined from the flow data shown in E and F.

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