Data Sheet

THP-1 NF-κB-eGFP Human Acute Monocytic Leukemia Cell Line

Cancer Cell Line

SCC223

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Microbial contamination of biological cultures is a universal concern for laboratories. Detection and monitoring of microbial contamination often require a significant commitment of time, labor and expense, especially when maintaining multiple cultures and preserving precious specimens. Methods that are at once efficient, cost-effective, and sensitive for a range of microbial contamination are of great value in facilitating detection and ensuring contamination-free cultures.

The THP-1 NF-κB-eGFP cell line makes use of the toll-like receptor (TLR) signaling pathway as a simple and sensitive biological assay for detection of microbial contamination. The suite of TLR proteins recognize distinct molecular signatures conserved across bacterial species and activate the transcription factor NF-κB. THP-1 NF-κB-eGFP cells exhibit high sensitivity for mycoplasma and gram-negative bacterial LPS.[1] Incubation of THP-1 NF-κB-eGFP cells for 24 hours with contaminated culture supernatants activates expression of the eGFP reporter which may be easily detected via flow cytometry or light microscopy.[1] Presence of amphotericin B in cultures also induces the reporter gene. This unique cell line is a simple method for detecting a range of common microbial contaminants and is suitable for both routine assays and high throughput screening of biological cultures.

Source

The THP-1 NF-κB-eGFP cell line is derived from a single-cell clone of THP-1 cells stably transfected with an NF-κB-eGFP reporter construct.[1] The parental THP-1 cell line was derived from peripheral blood of a 1-year-old male suffering from acute monocytic leukemia.[2]

Short Tandem Repeat

D3S1358: 15, 17	D16S539: 11, 12
TH01: 8, 9.3	CSF1PO: 11, 13
D21S11: 30, 31.2	Penta D:
D18S51: 13, 14	vWA: 16
Penta E: 11, 15	D8S1179: 10, 14
D5S818: 11, 12	TPOX: 8, 11
D13S317: 13	FGA: 24, 25
D7S820: 10	Amelogenin: X, Y

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.

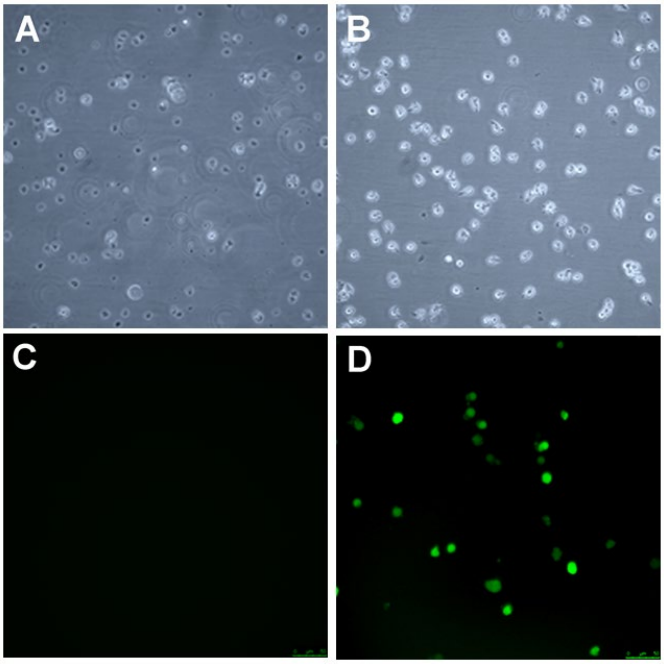
Quality Control Testing

- 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 rat, mouse, 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.
- Each lot of cells is genotyped by STR analysis to verify the unique identity of the cell line.

Storage and Handling

Store 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.

Presentation



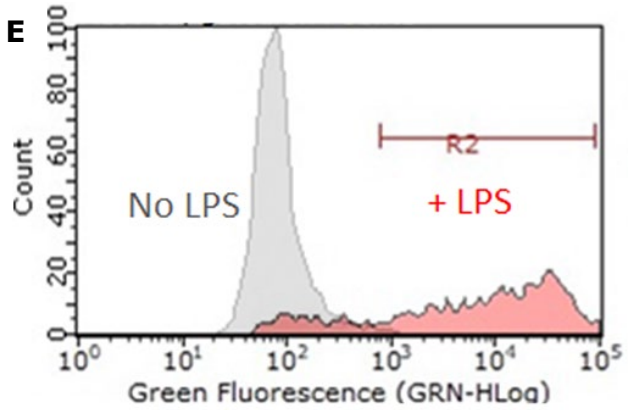


Figure 1. (**A**) Days one and (**B**) five after thaw. (**C**) No LPS control. (**D**) Activation of GFP fluorescence by exogenously applied lipopolysaccharide (LPS, 3 µg/mL). (**E**) Flow analysis.

Protocols

THP-1 NF-κB-eGFP cells grow as suspension cells and thus do not require enzymatic detachment or dissociation. Passage when the cell density reaches 1-1.5 million cells/mL. Optimal plating density should be ~400,000 cells/mL. The cells should not be grown at excessively high densities. Do not add amphotericin B to the culture medium as this will induce the reporter gene.

1. Do not thaw the cells until the recommended medium is on hand.
2. THP-1 NF-κB-eGFP Expansion Medium: cells are thawed and expanded in RPMI-1640 (R0883) and 10% heat-inactivated FBS (ES-009-B).
3. Remove the vial of frozen THP-1 NF-κB-eGFP 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.
4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
5. In a laminar flow hood, use a 1-2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
6. Using a 10 mL pipette, slowly add dropwise 9 mL of THP-1 NF-κB-eGFP 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.
7. 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.
8. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
9. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
10. Resuspend the cells in 5 mL of THP-1 NF-κB-eGFP Expansion Medium.
11. Transfer the cell suspension to a T25 flask.

12. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2. Place the flask upright for 3 days. Thawed cells may be slow to proliferate the first 3 days. From days 5-6, cell density will double. Passage cells when the cell density is at 1-1.5 million cells/mL.
13. Replenish the media every 2-3 days. Optimal plating density should be ~400,000 cells/mL

Cryopreservation of Cells

THP-1 NF-κB-eGFP Human Acute Monocytic Leukemia Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. PLOS One. 2017; 12(5): e0178220
2. Int J Cancer. 1980; 26(2): 171-176.

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