Data Sheet

Jurkat JE6.1 NF-κB::eGFP hTLR4 (Human Toll-like Receptor 4) Cell Line

SCC630

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

Contamination with bacterial components is a potential issue with the biologics for research and therapeutic uses, as they can activate the so-called pattern-recognition receptors in mammalian cells and trigger immune responses, clouding the experimental results or even causing health hazards. Such responses are often mediated by the Toll-like Receptors (TLRs), which recognize bacterial molecular patterns to activate, among other things, cytokine expression through the NF-κB pathway.[1,2]

The current assessment method for bacterial contamination measures lipopolysaccharide (LPS), the active component of endotoxins. Endotoxin measurement relies on its reaction with the limulus amoebocyte lysate (LAL), derived from the horseshoe crab blood.[3] While the method is well established, however, the use of primary lysate obtained from wild animals presents a major sustainability issue.[4] Recombinant alternatives to the primary LAL method have recently been gaining acceptance, but all LAL-based methods measure only the endotoxin contamination, and other classes of bacterial components go undetected.

To address these unmet needs, the Jurkat JE6.1 cell line has been engineered to form a group of cell lines, each expressing a specific set of TLRs and harboring an eGFP expression cassette fused to the NF-κB Response Element. The result is that each of these strains expresses eGFP in response to a specific class of bacterial molecules.[1,5]

Jurkat JE6.1 NF-κB::eGFP hTLR4 cell line (SCC630), expresses recombinant TLR4 and its co-receptors CD14 and MD-2 on its surface and is designed to express eGFP in response to LPS. It is not responsive to other bacterial components, such as the Gram positive- and Gram negative flagellins (ligands for SCC631, the TLR5 cell line), and FSL-1 (ligand for SCC632, the TLR2/TLR6 cell line). It has also been tested negative for PAM3CSK4 (ligand for TLR2/TLR1).[1]

Samples containing high concentration of LPS can be assayed by their serial dilution in the growth medium, and the limit of detection in such experiments is in the range of 0.5 pg/mL (0.005 EU/mL). Samples of low LPS content may be assayed by their direct addition to the growth medium in volumes not exceeding 10% of the medium, and the limit of detection in such reactions is in the range of 5 pg/mL (0.05 EU/mL). Only water and Dubecco's phosphate-buffered saline (DPBS) have been validated as the solvents for this purpose, but other vehicles may be appropriate. If components other than LPS are suspected of the resulting eGFP activation, the SCC635 cells (the non-responder cell line) can be used as a negative control.

Source

The Jurkat JE6.1 NF-κB::eGFP Cell Line (SCC635), the parental cell line for Jurkat JE6.1 NF-κB::eGFP hTLR4 cell line (SCC630), was made by the deletion of the TLR5 gene in the Jurkat JE6.1 cell line harboring the eGFP expression cassette fused to the NF-κB Response Element.[1,5] The Jurkat JE6.1 NF-κB::eGFP Cell Line (SCC635) was retrovirally transduced with the recombinant hTLR4, hCD14, hMD-2 to yield Jurkat JE6.1 NF-κB::eGFP hTLR4 cell line (SCC630).

Short Tandem Repeat

D3S1358: 15, 17	D7S820: 8, 11	vWA: 18, 19, 20	FGA: 20, 21	D8S1179: 13, 14
D21S11: 32.2	D18S51: 13, 21, 22	D5S818: 9	D13S317: 8, 11	D16S539: 11
TH01: 6, 9.3	TPOX: 8, 10	CSF1PO: 11	AMEL: X	Penta D: 11, 13
Penta E: 10, 12	Mouse: NA			

Quality Control Testing

- The Jurkat JE6.1 NF-κB::eGFP hTLR4 cell line is verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

The Jurkat JE6.1 NF-κB::eGFP hTLR4 cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

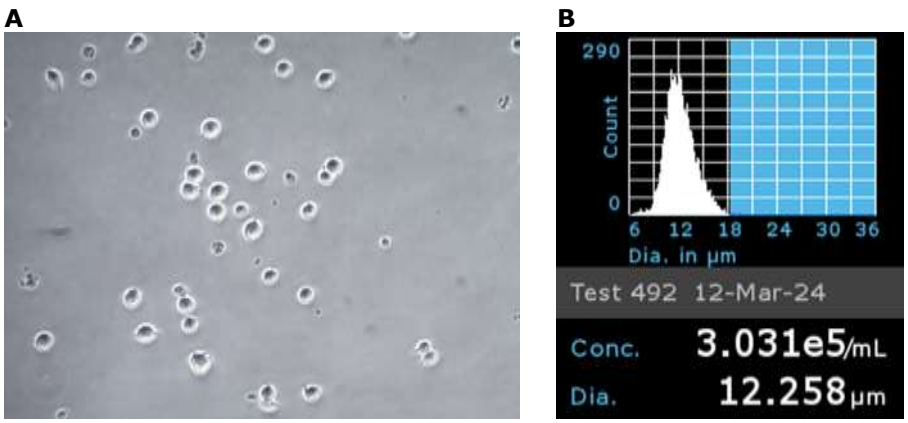

Figure 1. (**A.**) Bright-field image of Jurkat JE6.1 NF-κB::eGFP hTLR4 cells (SCC630), a day after thaw in a T75 flask. (**B.**) Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensor tips (PHCC360KIT).

Note: Product catalog numbers indicated in () can be purchased at SigmaAldrich.com unless otherwise stated.

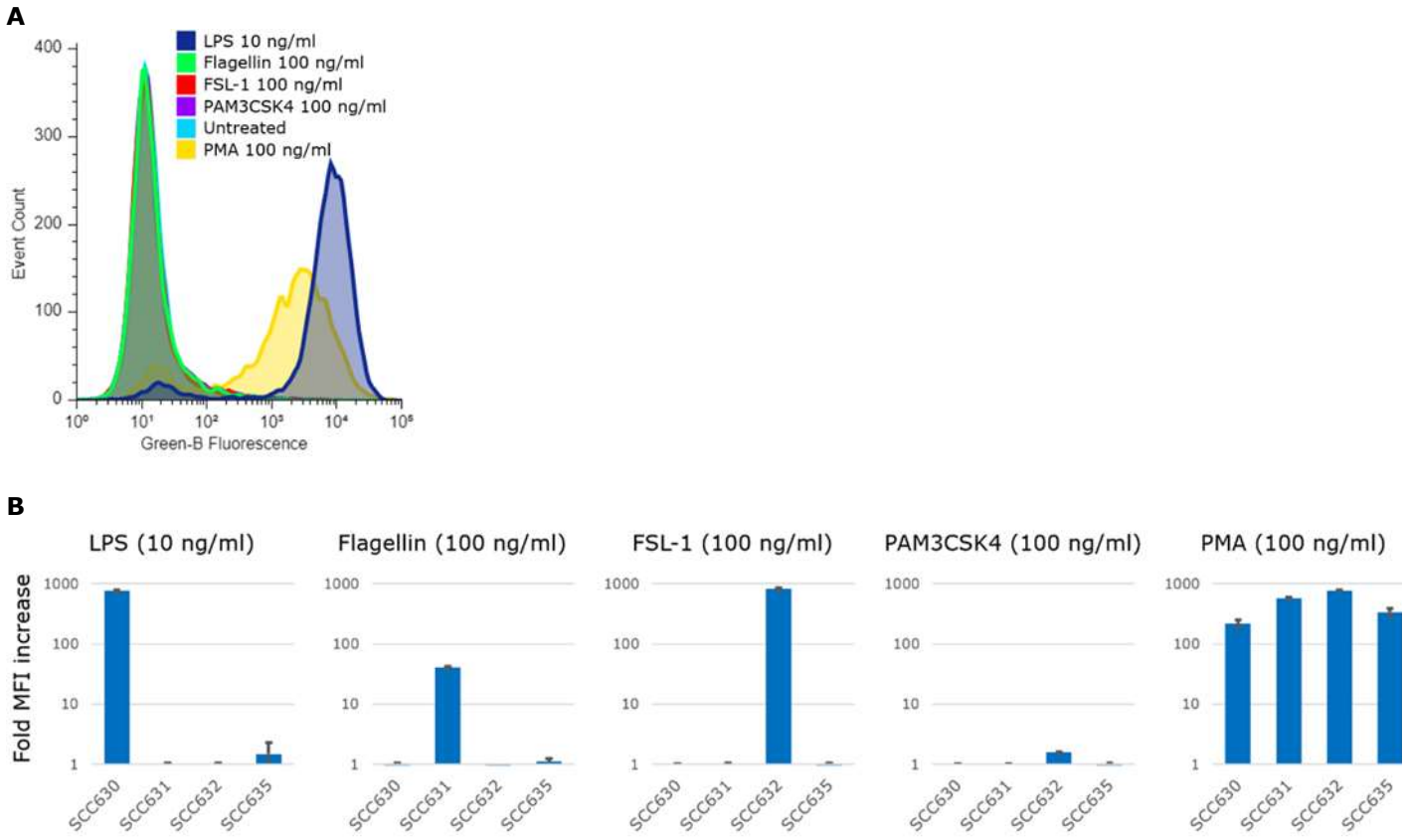

Figure 2. (**A**) The Jurkat JE6.1 NF-κB::eGFP hTLR4 cells express eGFP in response specifically to LPS. 50,000 cells were cultured on a 96-well plate in 100 µL expansion media containing the bacterial components for 24 hours and analyzed for eGFP expression by Guava easyCyte™ HT flow cytometer. (**B**) The Jurkat JE6.1 NF-κB::eGFP hTLR4 cells expresses eGFP minimally with other bacterial components at the concentrations that activate the cell lines that express the appropriate TLRs (TLR5 in SCC631, TLR2/TLR6 in SCC632). PMA, the chemical activator of the NF-κB pathway, was included as positive control. PAM3CSK4 is a ligand for the TLR2/TLR1 complex. The response is expressed in fold increase in median fluorescence intensity (MFI) over the basal eGFP expression. Each bar represents the biological triplicates +/- standard deviation.

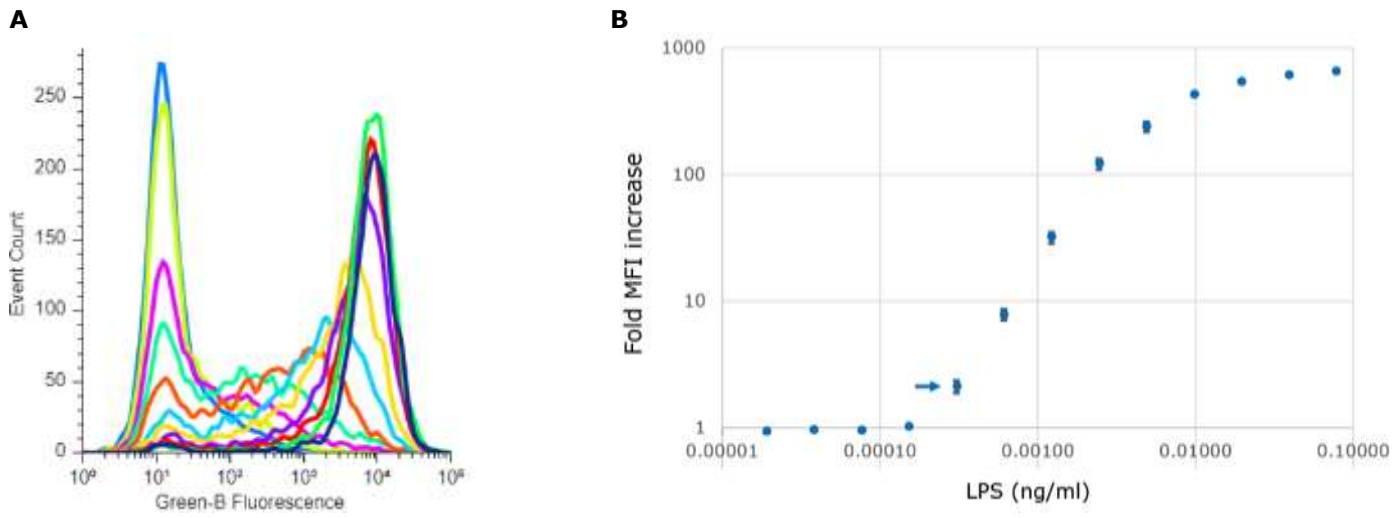

Figure 3. (**A**) LPS detection limit for concentrated samples. 50,000 cells were seeded on a 96-well plate with 100 µL expansion media and stimulated by LPS at the indicated concentrations for 24 hours and analyzed for eGFP expression by Guava easyCyte™ HT flow cytometer. The LPS gradient was formed by 2-fold serial dilutions. (**B**) Overlay of the histograms acquired at LPS concentrations between 80 pg/mL and 80 fg/mL. Only one representative histogram at each concentration point is shown for clarity. The detection limit, the lowest concentration point that achieved the median fluorescence intensity (MFI) increase of 1.5-fold over the unstimulated samples, was 0.3 pg LPS/mL, or 0.003 EU/mL (blue arrow; the two-tailed t-test p-value vs unstimulated sample=0.013). Each point represents a biological triplicate +/- standard deviation.

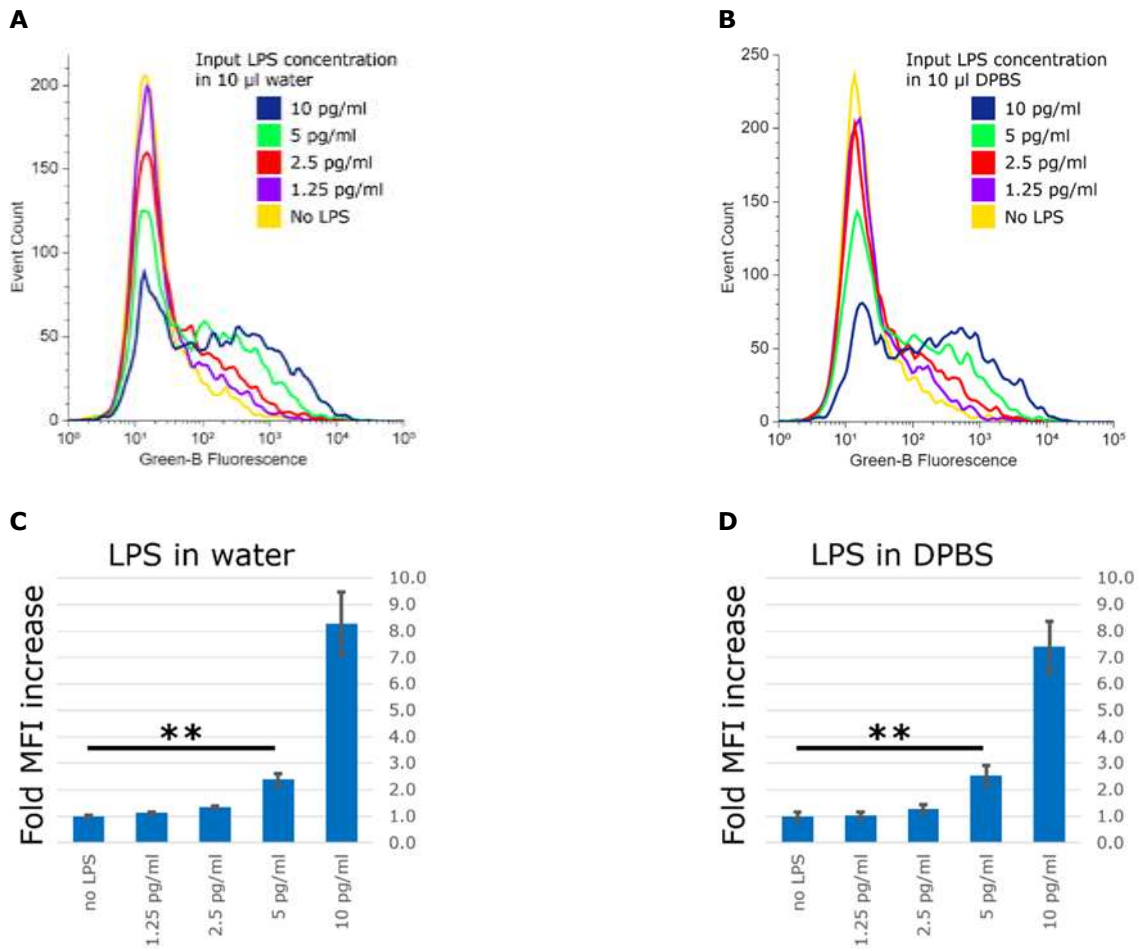

Figure 4. LPS detection by the direct addition of dilute samples. 50,000 cells were seeded on a 96-well plate with 90 µL expansion media and 10 µL of LPS solutions of the indicated concentrations for 24 hours and analyzed for eGFP expression by Guava easyCyte™ HT flow cytometer. (**A**) The LPS stock solutions were prepared in water or (**B**) Dulbecco's phosphate-buffered saline (DPBS). (**C**) The LPS concentration of the 10 µL input solution at the detection limit, the lowest concentration point that achieved the median fluorescence intensity (MFI) increase of 1.5-fold over the unstimulated samples, was 5 pg LPS/mL, or 0.05 EU/mL for water and (**D**) DPBS. The two-tailed t-test p-value vs unstimulated sample was below 0.01 for both. (**C**, **D**) Each bar represents a biological triplicate +/- standard deviation. In (**A**, **B**), only one representative histogram of the triplicate at each concentration is shown for clarity.

Protocols

Thawing the Cells

Do not thaw the cells until the recommended medium is on hand. The Jurkat JE6.1 NF-κB::eGFP hTLR4 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 ~200,000-250,000 cells/mL. The cells should not be grown at excessively high densities.

1. The Jurkat JE6.1 NF-κB::eGFP hTLR4 cells are thawed and expanded in The Jurkat JE6.1 NF-κB::eGFP hTLR4 cells Expansion Medium comprising of RPMI1640 with L-glutamine and sodium bicarbonate (R8758-500ML) containing 10% FBS (ES-009-B, heat-inactivated at 57 °C for 30 min) with optional Penicillin/Streptomycin (P4333).
2. Remove the vial of frozen 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 1-2 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 SCC630 Expansion Medium (medium composition in Step 1) to the 15 mL conical tube.

   IMPORTANT: The Expansion Medium should be pre-warmed to 37 °C. 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 200 x *g* for 3 minutes to pellet the cells.

   IMPORTANT: Higher spin speed and/or longer centrifugation may result in decreased viability.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative.
9. Resuspend the cells in 15 mL of the Jurkat JE6.1 NF-κB::eGFP hTLR4 cells Expansion Medium.
10. Transfer the cell mixture to a T25 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.
12. Cells are typically subcultured at the density of 200,000-250,000 cells/mL.

Cryopreservation of the Cells

The Jurkat JE6.1 NF-κB::eGFP hTLR4 cells may be frozen in the Expansion Medium with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

Related Products

SCC631, Jurkat JE6.1 NF-kB::eGFP hTLR5 Expressing Cell Line

SCC632, Jurkat JE6.1 NF-kB::eGFP hTLR2/6 Expressing Cell Line

SCC635, Jurkat JE6.1 NF-kB::eGFP Cell Line (Control)

References

1. Radakovics K, Battin C, Leitner J, Geiselhart S, Paster W, Stöckl J, Hoffmann-Sommergruber K, Steinberger P. 2022. A highly sensitive cell based TLR reporter platform for the specific detection of bacterial TLR ligands. Front Immunol. 12:817604.
2. Duan T, Du Y, Xing C, Wang HY, Wang R-F. 2022. Toll-like receptor signaling and its role in cell-mediated immunity. Front Immunol. 13:812774.
3. Tamura H, Reich J, Nagaoka I. 2021. Outstanding contributions of LAL technology to pharmaceutical and medical science: review of methods, progress, challenges, and future perspectives in early detection and management of bacterial infections and invasive fungal diseases. Biomedicines. 9(5):536.
4. Gorman R. 2020. Atlantic horseshoe crabs and endotoxin testing: perspectives on alternatives, sustainable methods, and the 3Rs (Replacement, Reduction, and Refinement). Front Mar Sci. 7:fmars.2020.582132.
5. Jutz S, Hennig A, Paster W, Asrak Ö, Dijanovic D, Kellner F, Pickl WF, Huppa JB, Leitner J, Steinberger P. 2017. A cellular platform for the evaluation of immune checkpoint molecules. Oncotarget. 8(39):64892-64906.

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