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Data Sheet

Jurkat JE6.1 NF-κB::eGFP hTLR2/6 (Human Toll-like Receptor 2&6) Cell Line

SCC632

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 hTLR2&6 cell line (SCC632), expresses recombinant TLR2 and TLR6 on its surface and is designed to express eGFP in response diacyl lipopeptides such as FSL-1. It is not responsive to other bacterial components, such as LPS (ligand for SCC630, TLR4 expressing), and Flagellin (ligand for SCC631, TLR5 expressing). It has also been tested negative for PAM3CSK4 (ligand for TLR2/TLR1).[1]

Source

The Jurkat JE6.1 NF-κB::eGFP hTLR2/6 (Human Toll-like Receptor 2&6) Cell Line (SCC632) was retrovirally transduced with TLR2. TLR2 and the endogenous TLR6 form functional complexes. Homodimers of TLR2 or TLR6 do not react with the commonly tested bacterial components, and the strain is specific to the TLR2/TLR6 ligands.

MilliPORE SiGMa

Short Tandem Repeat

D3S1358: 15, 17	D7S820: 8, 11	vWA: 18, 19	FGA: 20, 21	D8S1179: 13, 14
D5S818: 9	D13S317: 8, 11	D16S539: 11	TH01: 6, 9.3	TPOX: 8, 10
CSF1PO: 11	AMEL: X, Y	Penta D: 11, 13	Penta E: 10, 12	Mouse: NA
D21S11: 31.2, 32.2		D18S51: 13, 20, 21, 22		

Quality Control Testing

- The Jurkat JE6.1 NF-κB::eGFP hTLR2&6 Cell Line (SCC632) are 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 hTLR2&6 Cell Line (SCC632) 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

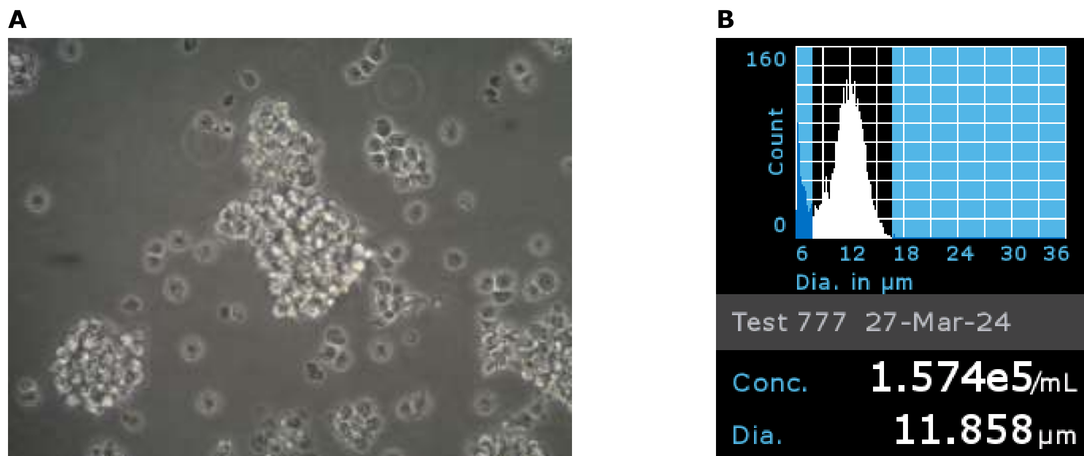

Figure 1. (**A**) Bright-field image of Jurkat JE6.1 NF-κB::eGFP hTLR2&6 cell line (SCC632) a day after thaw in a T25 flask. (**B**) Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensor tips (PHCC360KIT).

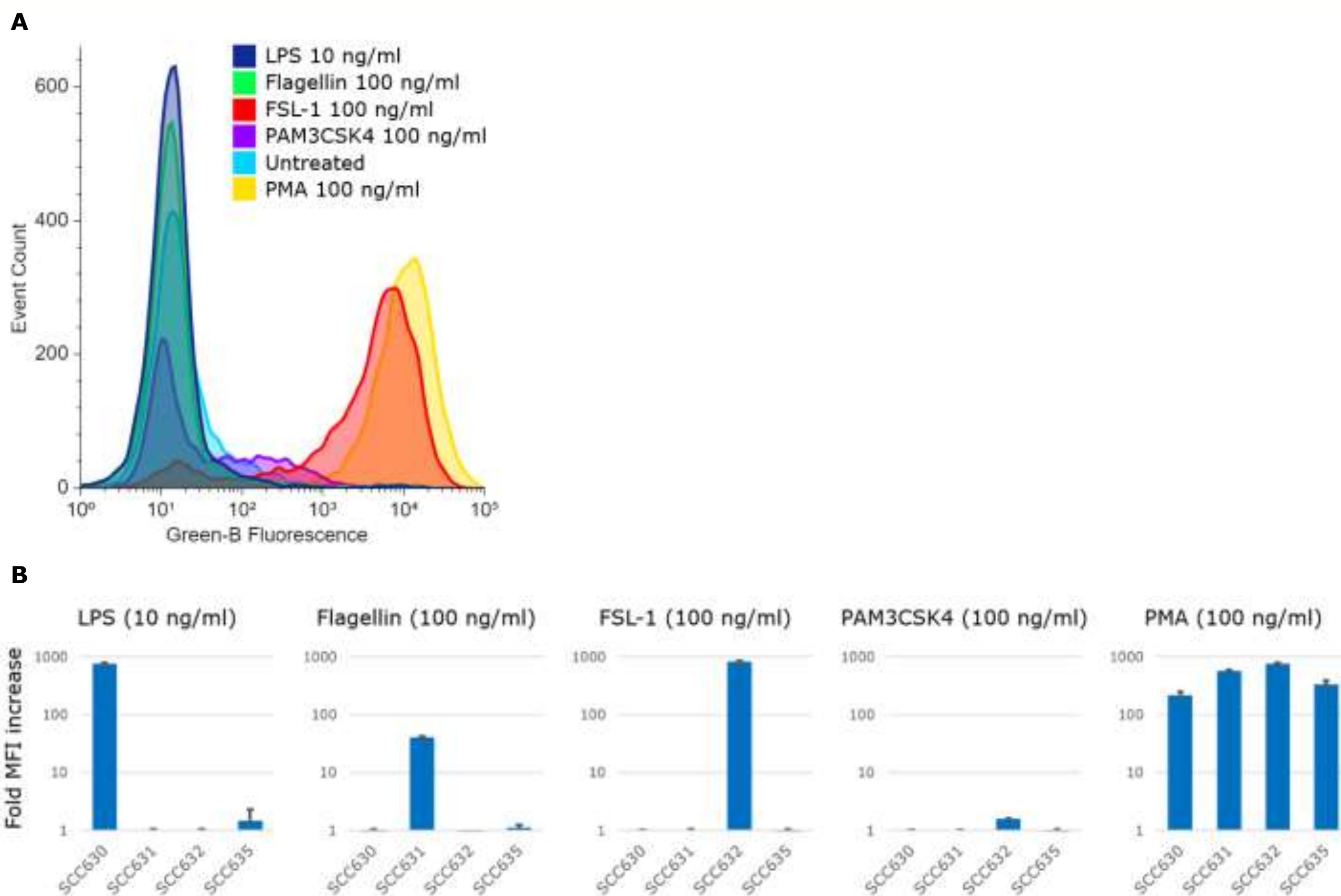

Figure 2. The Jurkat JE6.1 NF-κB::eGFP hTLR2&6 cell line (SCC632) express eGFP in response specifically to FSL-1. (**A**) 50,000 cells were cultured on a 96-well plate in 100 µL expansion media containing the bacterial components for 24-hours and analyzed for eGFP expression by Guava® easyCyte HT flow cytometer. The Jurkat JE6.1 NF-κB::eGFP hTLR2&6 cell line (SCC632) expresses eGFP minimally with other bacterial components at the concentrations that activate the cell lines that express the appropriate TLRs (TLR4 in SCC630, TLR5 in SCC631), as shown in (**B**). 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.

Protocols

Thawing the Cells

Do not thaw the cells until the recommended medium is on hand. The Jurkat JE6.1 NF-κB::eGFP hTLR2&6 cell line (SCC632) 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 hTLR2&6 Cell Line (SCC632) are thawed and expanded in SCC632 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 SCC632 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 SCC632 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 sub cultured at the density of 200,000-250,000 cells/mL.

Cryopreservation of the Cells

The Jurkat JE6.1 NF-κB::eGFP hTLR2&6 Cell Line (SCC632) may be frozen in the expansion medium with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

Related Products

SCC630, Jurkat JE6.1 NF-κB::eGFP hTLR4 Expressing Cell Line

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

SCC635, Jurkat JE6.1 NF-κB::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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