Data Sheet

Jurkat JE6.1 NF-κB::eGFP hTLR2 (Human Toll-like Receptor 2)

Cell Line

SCC634

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 horseshoe crab blood.[3] While the method is well established, 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 T NF-κB::eGFP hTLR2 cell line (SCC634) expresses recombinant TLR2 on its surface and is designed as a control which only expresses eGFP after treatment with PMA. It is not responsive to other bacterial components, such as LPS (ligand for SCC630, TLR4 expressing), and FSL-1 (ligand for SCC632, the TLR2/TLR6 cell line). It has also been tested negative for PAM3CSK4 (ligand for TLR2/TLR1). These results indicate that TLR2 heterodimers are required for recognition of TLR2 related ligands.[1]

Source

Genetically Modified Organisms (GMO)

Jurkat JE6.1 was retrovirally (pBABE-MN) transduced with the NF-κB reporter construct, which is activated only by PMA treatment to serve as a control.

Short Tandem Repeat

D3S1358: 15, 17	D7S820: 8, 11	vWA: 18, 19	FGA: 20, 21	D8S1179: 13, 14
D5S818: 9	D13S317: 8, 10, 11	D16S539: 11, 12	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		

Quality Control Testing

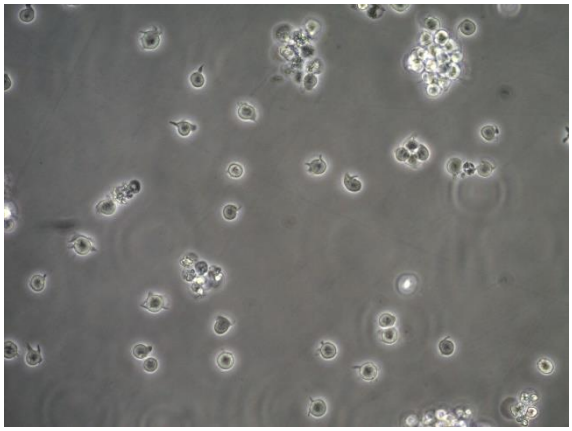
- SCC634 cells are verified to be of human origin and negative for mouse, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

SCC634 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

A



B

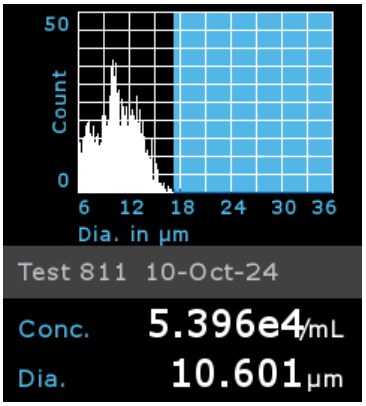


Figure 1: (**A**) Bright-field image of SCC634 cells a day after thaw in a T25 flask. (**B**) Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensor tips (PHCC360KIT).

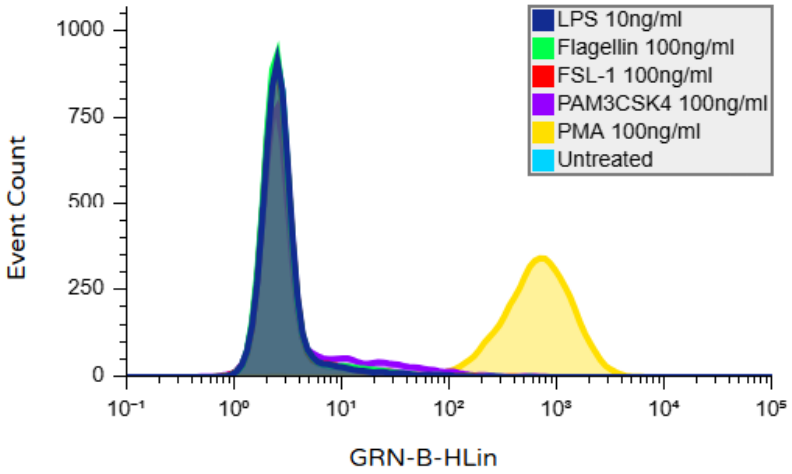


Figure 2: SCC634 cells express eGFP in response only to PMA treatment as a positive control. 500,000 cells were cultured on a 12-well plate in 1 mL Expansion Media containing the bacterial components for 24-hours and analyzed for eGFP expression by Guava® EasyCyte HT Flow Cytometer. SCC634 expresses eGFP minimally with all bacterial components at the concentrations that activate the cell lines that express the appropriate TLRs (TLR4 in SCC630, TLR2/TLR6 in SCC632, TLR5 in SCC631, and TLR2/1 in SCC633).

Protocols

Thawing the Cells

Do not thaw the cells until the recommended medium is on hand. SCC634 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. SCC634 cells are thawed and expanded in SCC634 Expansion Medium comprising of RPMI1640 with L-glutamine and sodium bicarbonate (R8758-500ML) containing 10% FBS (ES-009-B) 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 SCC634 Expansion Medium (medium composition in Step 1) to the 15 mL conical tube.

   IMPORTANT: The expansion medium should be pre-warmed to 37 °C and 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 10 mL of SCC634 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

SCC634 cells 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.

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

SCC633, Jurkat JE6.1 NF-κB::eGFP hTLR2/1 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.

Academic Use Agreement

Subject to local law

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means Jurkat JE6.1 NF-κB::eGFP hTLR2 (Human Toll-like Receptor 2) (SCC634).

"Academic Research" means any internal in vitro research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST

Genetically Modified Organisms (GMO)

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document Template 20306518 Ver 6.0
23203100 Ver 1.0, Rev 01APR2025,AV