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Product Sheet

H_TLR4 Reporter 293 Cell Line

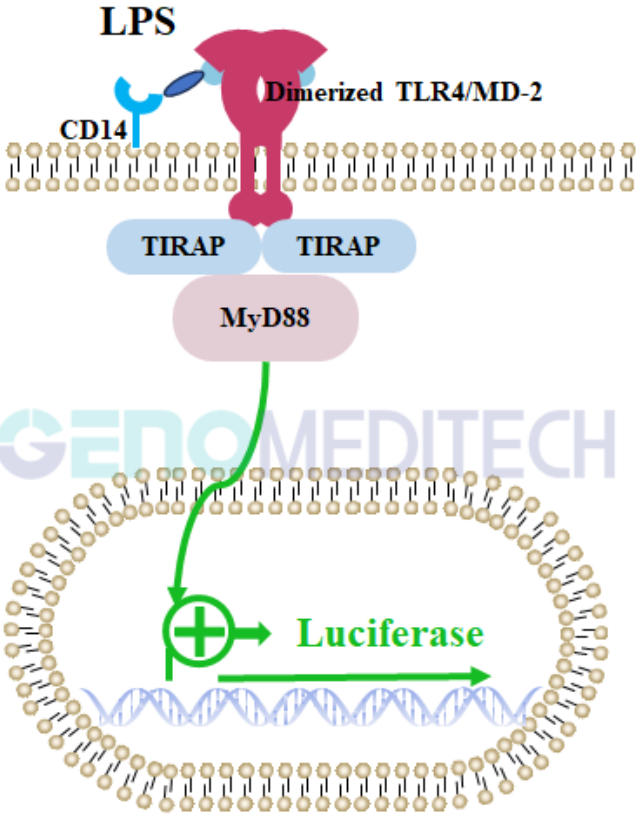
Catalog number: GM-C50021

Version 4.0.26.1014

TLR4 (Toll-like receptor 4) is a key pattern recognition receptor in the immune system, recognizing pathogen-associated molecular patterns (PAMPs) like bacterial lipopolysaccharides (LPS). It is mainly expressed in macrophages and dendritic cells, and its activation triggers inflammatory responses, enhancing the body's resistance to infections.

TLR4 signaling occurs through MyD88-dependent and MyD88-independent pathways. Activation of TLR4 leads to MyD88 binding, initiating downstream signaling that activates transcription factors such as NF-κB and MAPK, promoting inflammatory factors like TNF-α and IL-1β. The MyD88-independent pathway, mediated by TRIF, activates the interferon regulatory factor (IRF) pathway, enhancing interferon production.

H_TLR4 Reporter 293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the TLR4 gene and some adapter membrane molecules, along with signal-dependent expression of a luciferase reporter gene. When LPS binds to TLR4, it activates downstream signaling pathways, leading to the expression of luciferase. The luciferase activity measurement indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of drugs related to TLR4.



Specifications

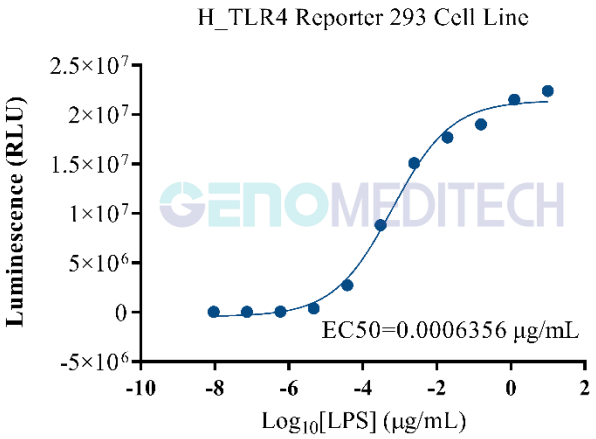
Quantity	5E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Recovery Medium	DMEM+10% FBS(Delipidized)+1% P.S
Growth medium	DMEM+10% FBS(Delipidized)+1% P.S+4 μg/mL Blasticidin+400 μg/mL G418+125 μg/mL Hygromycin+0.75 μg/mL Puromycin
Note	Use brand-matched serum and dissociation reagents as follows:Lipid Depleted Fetal Bovine Serum（VivaCell C3840）、CD-Trpizer® Enzyme-free Cell Dissociation Reagent（BasalMedia/S343JV）
Freezing Medium	90% FBS(Delipidized)+10% DMSO
Growth properties	Adherent
Growth Conditions	37°C, 5% CO_2
Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
DMEM	Gibco/C11995500BT
Lipid Depleted Fetal Bovine Serum	VivaCell/C3840
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
Hygromycin	Genomeditech/GM-040403
G418	Genomeditech/GM-040402
CD-Trpizer® Enzyme-free Cell Dissociation Reagent	BasalMedia/S343JV
96 well round cell culture plate	PakGent/CL-PT096
96-well White Opaque Plate	Thermo/236108
96-well U-bottom Plate	Saining/1014010
LPS	Beyotime/S1732
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040513

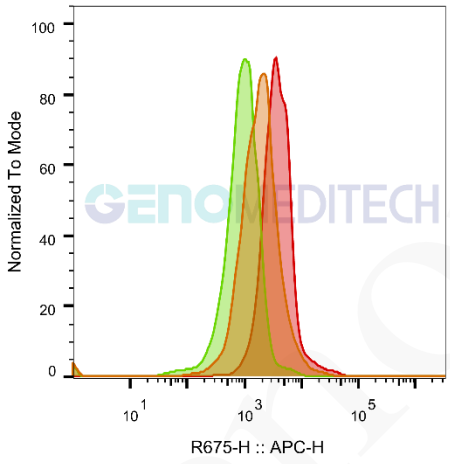
Data

Signaling Pathway



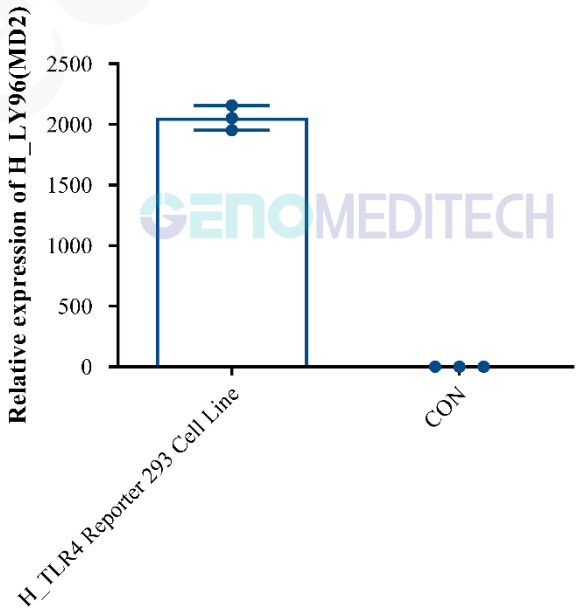
Response to LPS. H_TLR4 Reporter 293 Cell Line (Cat. GM-C50021) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, serial dilutions of the LPS (Beyotime/S1732) were added to the pre-seeded cells. The mixture was incubated for an additional 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [517.8]. Data are shown by drug mass concentration.

Expression

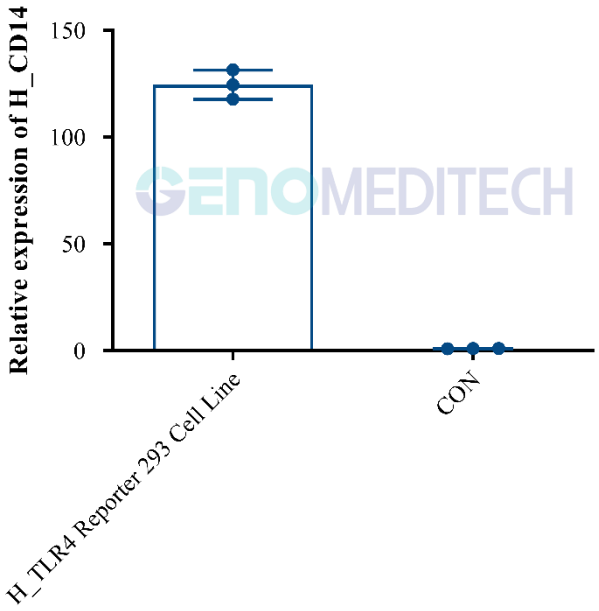


	SampleID	Geometric Mean : R675-H
	293 APC anti-human TLR4 Ab	1742
	H_TLR4 Reporter 293 APC M_IgG TLR4 Ab	869
	H_TLR4 Reporter 293 APC anti-human TLR4 Ab	3646

H_TLR4 Reporter 293 Cell Line (Cat. GM-C50021) was determined by flow cytometry using APC anti-human CD284 (TLR4) Antibody (biolegend/312815).



The mRNA expression levels of H_LY96(MD2) in the H_TLR4 Reporter 293 Cell Line (Cat. GM-C50021) were determined by RT-Qpcr.



The mRNA expression levels of H_CD14 in the H_TLR4 Reporter 293 Cell Line (Cat. GM-C50021) were determined by RT-Qpcr.

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Cell Recovery

Recovery Medium: DMEM+10% FBS(Delipidized)+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium. And spin at approximately 176 x g for 5 minutes. Discard supernatant.
d) Resuspend cell pellet with the recommended complete medium. And dispense the suspension into an appropriate culture flask and initially place the flask in an upright position after thawing.
e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS(Delipidized)+10% DMSO

a) Centrifuge at 176 x g for 3 minutes to collect cells.
b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
c) Aliquot 1 mL into each vial.
d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: DMEM+10% FBS(Delipidized)+1% P.S+4 μg/mL Blasticidin+400 μg/mL G418+125 μg/mL Hygromycin+0.75 μg/mL Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

a) Remove and discard culture medium.
b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
c) Add 1.0 mL Enzyme-free cell dissociation solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 30 to 60 seconds at 37°C).
d) Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
g) Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 - 1:4 is recommended

Medium Renewal: Every 3 to 4 days

Notes

a) During the first 1–3 days post-thaw, the cells may fail to attach. If this occurs, supplement the medium with an additional 5% FBS and extend the observation period. Cells generally begin to attach by day 4, and the first passage can be performed on day 5. After one passage at a 1:2 split ratio, cell growth gradually recovers, reaching 80% confluency or higher within 2–3 days.

b) It is important to maintain the cell density below 80% confluence; otherwise, excessive crowding may lead to reduced cell viability due to compression stress.

c) FBS(Delipidized) should be heat-inactivated at 56°C for 30 minutes to inactivate complement proteins and certain viruses, without significantly affecting the activity of most growth factors and cytokines.

License Agreement

By purchasing and using this cell line product, the user voluntarily agrees to accept and abide by the following policies:

- This cell line product is restricted to research use only and shall not be used for any commercial purposes.
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