Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_TLR9 Reporter 293 Cell Line

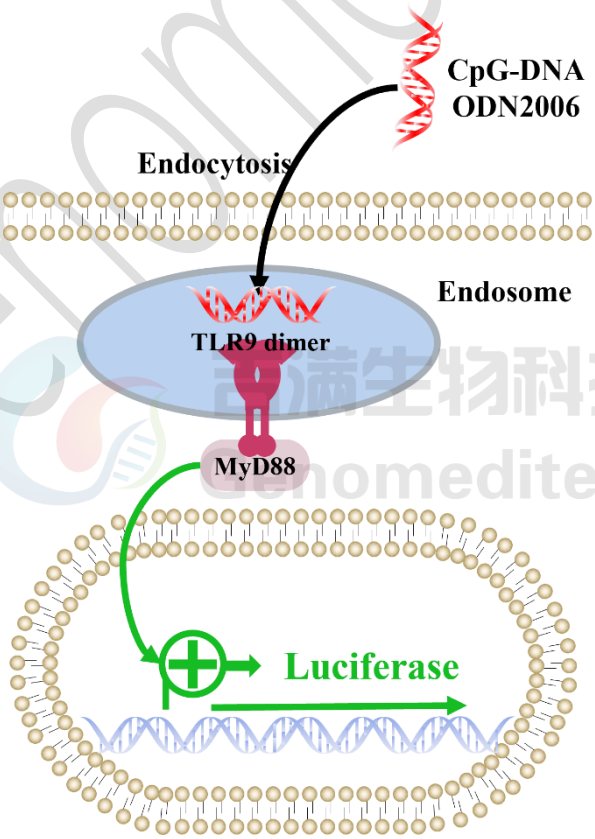
Catalog number: GM-C15428

Version 3.3.1.241216

TLR9 (Toll-like receptor 9) is an intracellular pattern recognition receptor that primarily detects unmethylated double-stranded DNA (dsDNA), especially from viruses and bacteria. It is expressed in immune cells like dendritic cells, B cells, and macrophages, contributing to both innate and adaptive immune responses by activating the immune system and promoting inflammation and interferon production.

The TLR9 signaling pathway is activated when unmethylated dsDNA binds to TLR9, recruiting the adaptor protein MyD88, which triggers downstream signaling. This pathway activates transcription factors such as NF-κB and IRF7, leading to the expression of interferons and other cytokines. Thus, TLR9 is crucial for regulating immune responses and antiviral defense.

H_TLR9 Reporter 293 Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutive expression of the TLR9 gene, along with signal-dependent expression of a luciferase reporter gene. When ODN 2006 binds to TLR9, 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 TLR9.



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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	EMEM(ATCC)+10% FBS+1% P.S
Growth medium	EMEM(ATCC)+10% FBS+1% P.S+3 μg/mL Blasticidin+1.5 μg/mL Puromycin
Note	Cells should be cultured using ATCC/30-2003 EMEM medium or Growth medium from Genomeditech. The serum should be Cegrogen biotech/A0500-3010 or sourced from Gibco.
Freezing Medium	90% FBS+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.
EMEM	ATCC/30-2003
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
ODN 2006	Sangon Biotech/
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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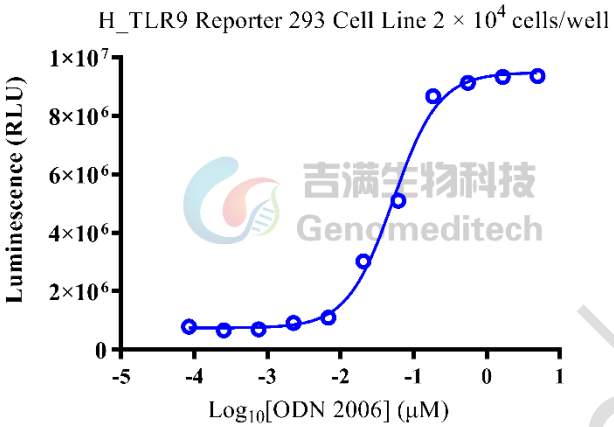
Figures



	H_TLR9 Reporter 293 Cell Line
EC50	0.05273

Figure 1 | Response to ODN 2006. H_TLR9 Reporter 293 Cell Line (Cat. GM-C15428) at a concentration of 2E4 cells/well (96-well format) was stimulated with serial dilutions of ODN 2006 (Sangon Biotech) in assay buffer (EMEM(ATCC) + 1% FBS + 1% P.S) for 24 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [25.6]. Data are shown by drug molar concentration.

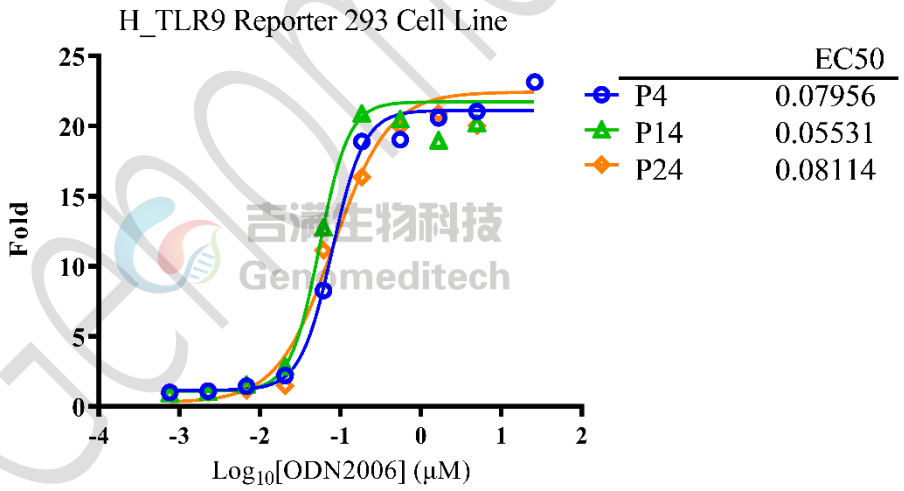


Figure 2 | The passage stability of response to ODN 2006. The passage 4, 14 and 24 of H_TLR9 Reporter 293 Cell Line (Cat. GM-C15428) at a concentration of 2E4 cells/well (96-well format) was stimulated with serial dilutions of ODN 2006 (Sangon Biotech) in assay buffer (EMEM(ATCC) + 1% FBS + 1% P.S) for 24 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug molar concentration.

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Cell Recovery

Recovery Medium: EMEM(ATCC)+10% FBS+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 recovery medium. And dispense into appropriate culture dishes.
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+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: EMEM(ATCC)+10% FBS+1% P.S+3 μg/mL Blasticidin+1.5 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 of 0.25% (w/v) Trypsin-EDTA 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

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Medium Renewal: Every 2 to 3 days

Notes

a) Upon initial revival, a higher number of dead cells and poor adherence are observed, which is normal. Adherence typically recovers within 2 - 3 days. After 2 - 3 passages, the proportion of adherent cells increases, and the cells begin to spread normally.

b) After each passage, there may be 5 - 10% dead cells; however, as the number of passages increases, the recovery rate accelerates, the proportion of dead cells decreases, and the cell growth rate stabilizes.

c) It is recommended to retain cell images after revival and during each observation to assist in assessing cell status. In case of abnormalities, promptly communicate with Genomeditech sales.

Related Products

TLR7	
H_TLR7 Reporter 293 Cell Line	Mouse_TLR7 Reporter 293 Cell Line
TLR9	
Mouse_TLR9 Reporter 293 Cell Line	
TLR8	
H_TLR8 Reporter 293 Cell Line	H_TLR8 HEK-293 Cell Line
STING	
H_STING KO THP1 Cell Line	H_STING KO U937 Cell Line
STING KO Reporter THP1 Cell Line	STING Reporter HEK-293 Cell Line
STING Reporter THP1 Cell Line	STING Reporter U937 Cell Line

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