

Product Sheet

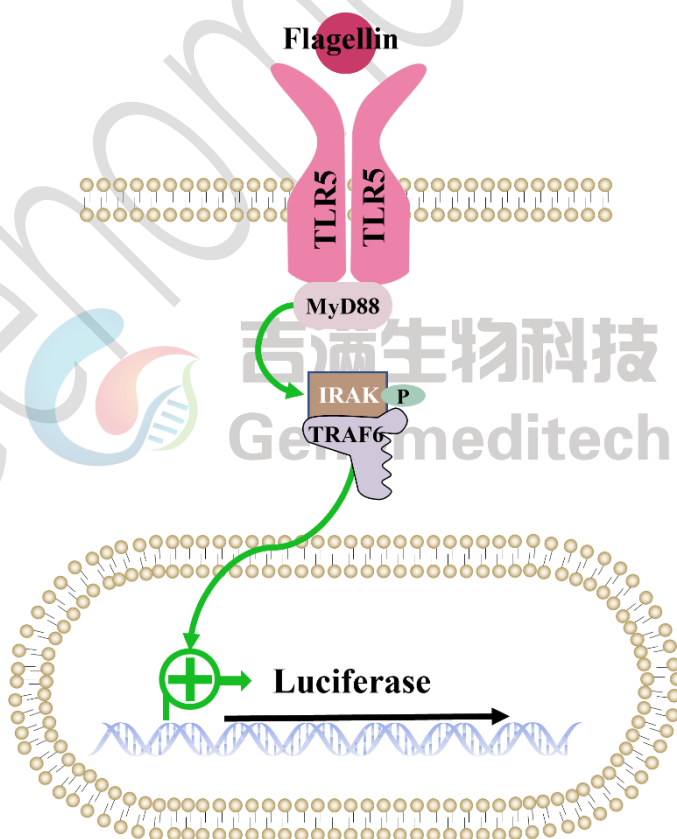
H_TLR5 Reporter 293 Cell Line

Catalog number: GM-C42128

Version 3.3.1.26.912

The TLR5 gene (Toll-Like Receptor 5) is an important component of the human innate immune system, located on chromosome 1q41–q42. It encodes a transmembrane receptor protein that primarily recognizes bacterial flagellin, thereby activating innate immune signaling pathways. Activation of TLR5 recruits downstream molecules such as MyD88, leading to activation of transcription factors like NF- κ B and the production of inflammatory cytokines, including IL-6 and TNF- α . TLR5 plays a crucial role in defending against bacterial infections, maintaining gut microbiota homeostasis, and regulating mucosal immunity. Genetic polymorphisms in TLR5 have been associated with susceptibility to diseases such as inflammatory bowel disease, obesity, diabetes, and infectious diseases, highlighting its essential physiological role in preserving immune balance and host defense.

H_TLR5 Reporter 293 Cell Line is a clonal stable 293 cell line constructed using lentiviral technology, constitutive expression of the human TLR5 gene, along with signal-dependent expression of a luciferase reporter gene. When the drug binds to the receptor, 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 be used to evaluate the in vitro effects of related drugs.



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+1% P.S
Growth medium	DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+0.75 µg/mL Puromycin
Note	None
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.
DMEM	Gibco/C11995500BT
Fetal Bovine Serum	ExCell/FSP500
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/ GM-040404
Puromycin	Genomeditech/ GM-040401
Flagellin Protein, <i>Listeria monocytogenes</i> (His)	MCE/HY-P75185
GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit	Genomeditech/ GM-040513

Figures

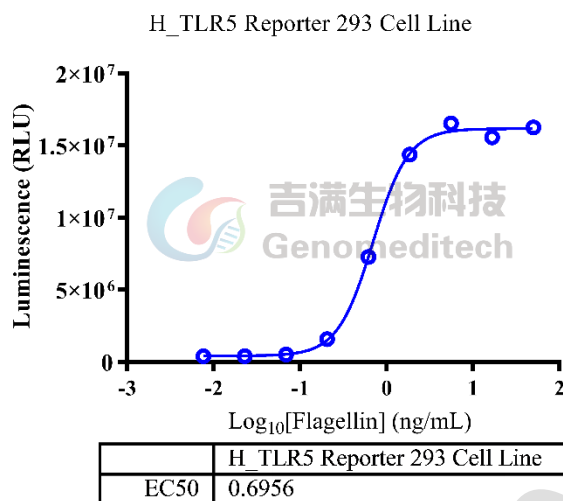


Figure 1 | Response to Flagellin. The H_TLR5 Reporter 293 Cell Line (Cat. GM-C42128) at a concentration of 2E4 cells/well (96-well format) was stimulated with serial dilutions of Flagellin, *Listeria monocytogenes* (His) (MCE/HY-P75185) in assay buffer (DMEM +1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [46.5]. Data are shown by drug mass concentration.

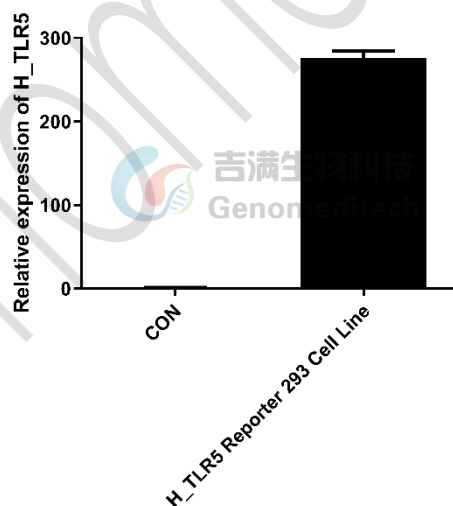


Figure 2 | The mRNA expression levels of H_TLR5 in the H_TLR5 Reporter 293 Cell Line (Cat. GM-C42128) were determined by RT-qPCR.

Cell Recovery

Recovery Medium: DMEM+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.

- 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).
- 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.
- 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.
- Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.
- Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

- Centrifuge at 176 x g for 3 minutes to collect cells.
- Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
- Aliquot 1 mL into each vial.
- 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+1% P.S+4 µg/mL Blasticidin+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.

- Remove and discard culture medium.
- Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
- 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).
- 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.
- Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
- After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
- 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) Cells that have just revived often show a relatively high proportion of dead cells, which is a normal phenomenon. After adjustment, the condition usually improves significantly. Once stabilized, the proportion of dead cells decreases after subculture, and the cell growth rate tends to become steady.
- b) After passaging, cells typically require 1–2 days to reattach. 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 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:

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