# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

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# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

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# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

## Human TLR3 (Luc) HEK293 Reporter Cell

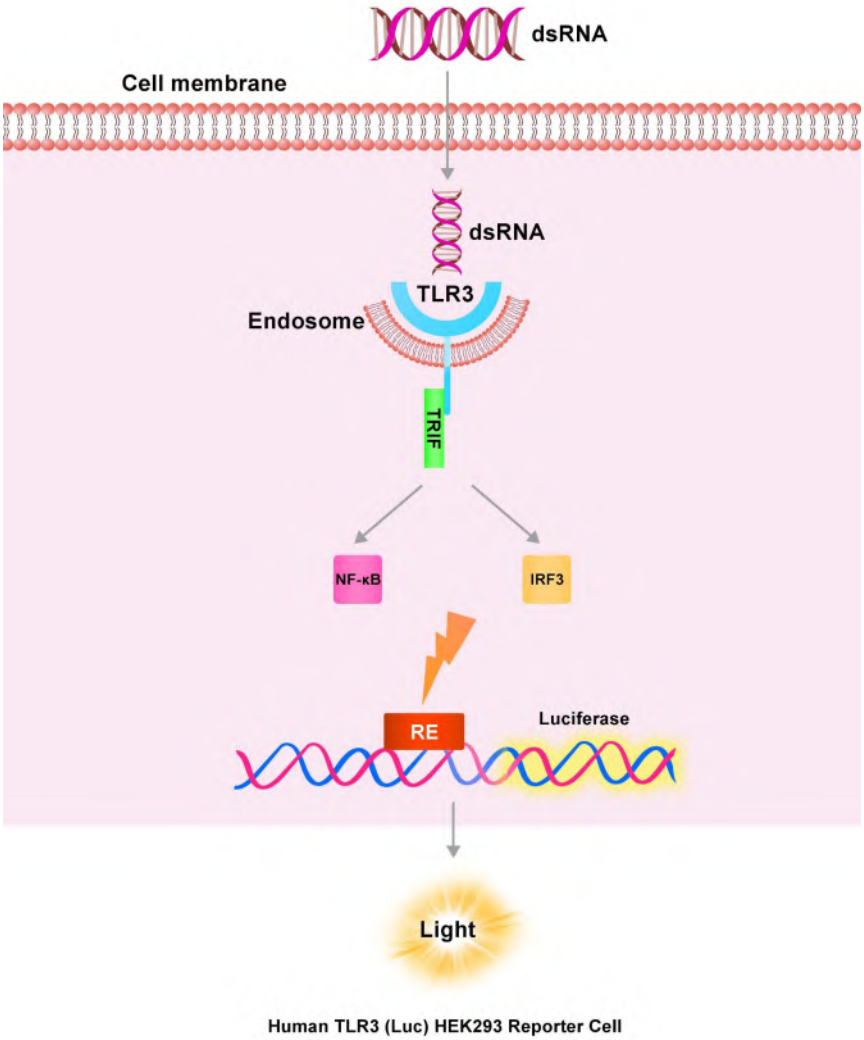
| Catalog No. | Size |
|---|---|
| CHEK-ATF309 | 2 × (1 vial contains ~5×10^6 cells) |

### • *Description*

The Human TLR3 (Luc) HEK293 Reporter Cell was engineered to not only express signaling response element driving luciferase expressing systems, but also express the receptor human TLR3 (Uniprot: O15455-1). When stimulated with dsRNA, receptor-mediated signaling can drive RE-mediated luminescence.

### • *Application*

- Detection of dsRNA




Human TLR3 (Luc) HEK293 Reporter Cell

# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

## • *Cell Line Profile*

| | |
|---|---|
| **Cell line** | Human TLR3 (Luc) HEK293 Reporter Cell |
| **Host Cell** | HEK293 |
| **Property** | Adherent |
| **Complete Growth Medium** | DMEM + 10% FBS |
| **Selection Marker** | Puromycin (2 μg/mL) + Hygromycin B (20 μg/mL) |
| **Incubation** | 37°C with 5% $CO_2$ |
| **Doubling Time** | 22-24 hours |
| **Transduction Technique** | Lentivirus |

## • *Materials Required for Cell Culture*

- DMEM Medium (BasalMedia, Cat. No. L120KJ)

**Note:** If you are unable to obtain the specified DMEM medium (BasalMedia, Cat. No. L120KJ), you may use an alternative DMEM medium (Gibco, Cat. No. 11965-092) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)

**Note:** For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: DMEM + 10% FBS, 1%P/S
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin B (20 μg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

## • *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37℃ with 5% $CO_2$ incubator until the cells are ready to be split.

## • *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:4 to 1:8 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37℃ with 5% $CO_2$ incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

**Note:**

(1) After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

(2) To ensure optimal cell health, it is essential to replace with a new T75 flask at each passage.

# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

## • *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of $5\times10^6$ to $1\times10^7$ cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

**Note:** It is recommended to establish a cell bank at the earliest possible passage for long-term use.

## • *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

• *Signaling Bioassay*

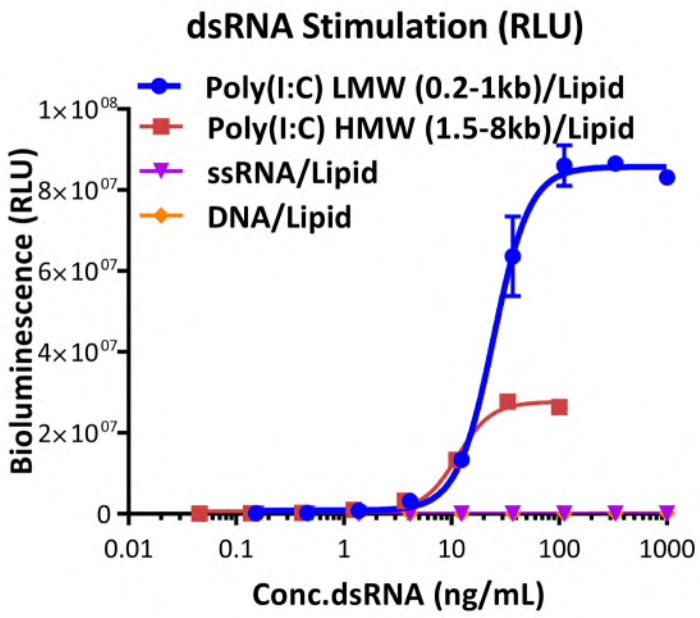



**Fig1. Response to dsRNA (RLU).** The Human TLR3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Poly(I:C) LMW (0.2-1kb), Poly(I:C) HMW (1.5-8kb), ssRNA and DNA complexed with Lipofectamine™ RNAiMAX. The reporter cell showed strong and dose-dependent activation in response to both Poly(I:C) LMW (0.2-1kb) and Poly(I:C) HMW (1.5-8kb), with EC50 of approximately 24.47 ng/mL and 11.31 ng/mL, respectively. In contrast, neither ssRNA nor DNA induced detectable luciferase activation across the tested concentration range (up to 1,000 ng/mL), demonstrating the dsRNA-specific responsiveness of this reporter cell.

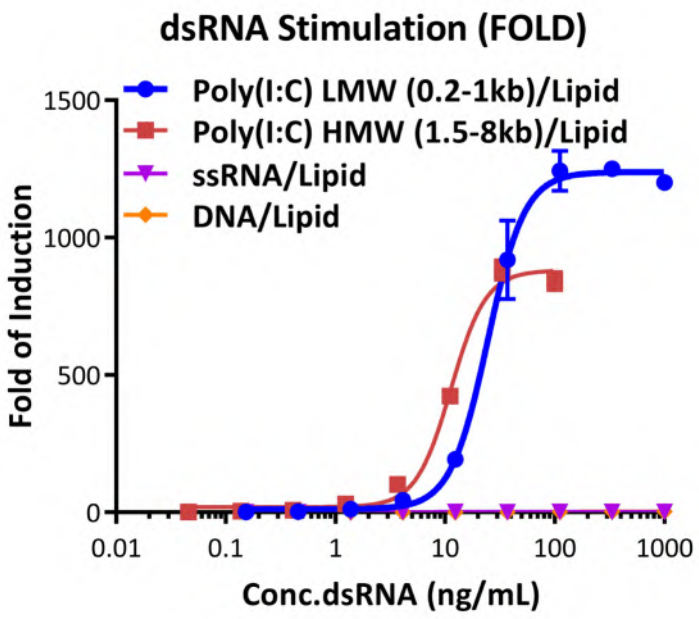



**Fig2. Response to dsRNA (FOLD).** The Human TLR3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Poly(I:C) LMW (0.2-1kb), Poly(I:C) HMW (1.5-8kb), ssRNA and DNA complexed with Lipofectamine™ RNAiMAX. The reporter cell showed strong and dose-dependent activation in response to both Poly(I:C) LMW (0.2-1kb) and Poly(I:C) HMW (1.5-8kb), with max induction folds of approximately 1250.01 and 882.35, respectively. In contrast, neither ssRNA nor DNA induced detectable luciferase activation across the tested concentration range (up to 1,000 ng/mL), demonstrating the dsRNA-specific responsiveness of this reporter cell.

# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

- ***Application***

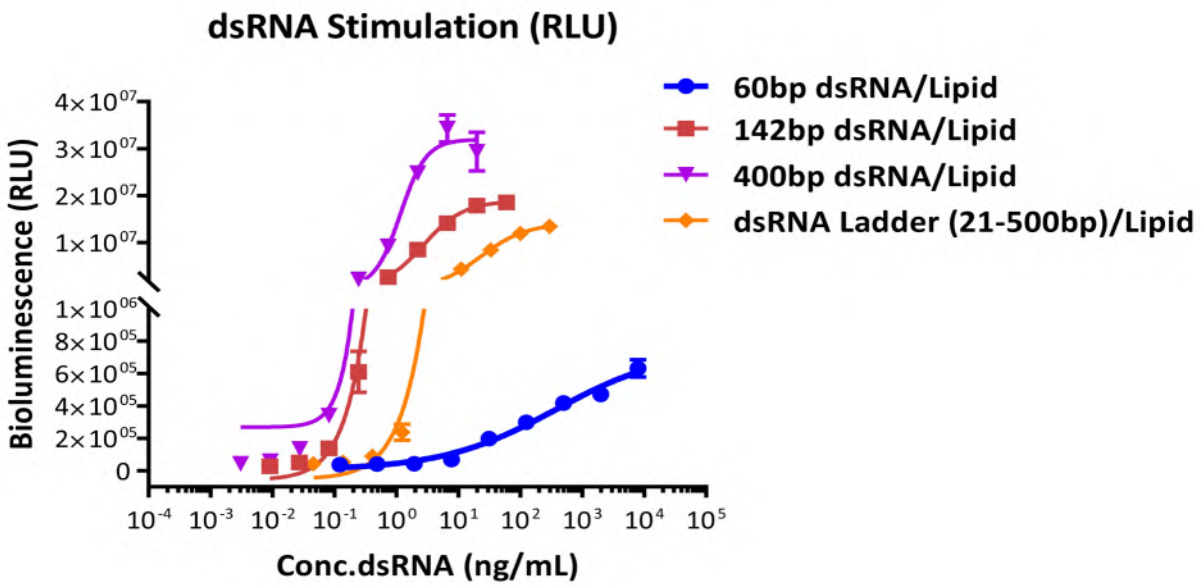



**Fig3. Bioactivity Detection of dsRNA with different lengths (RLU).** The Human TLR3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Lipofectamine™ RNAiMAX-complexed dsRNA samples with different lengths (60, 142, 400 bp) and a dsRNA ladder (21–500 bp). The induction fold exceeded 5 at approximately 32 ng/mL for 60 bp dsRNA, 0.09 ng/mL for 142 bp dsRNA, 0.09 ng/mL for 400 bp dsRNA, and 1.3 ng/mL for a dsRNA ladder (21–500 bp).

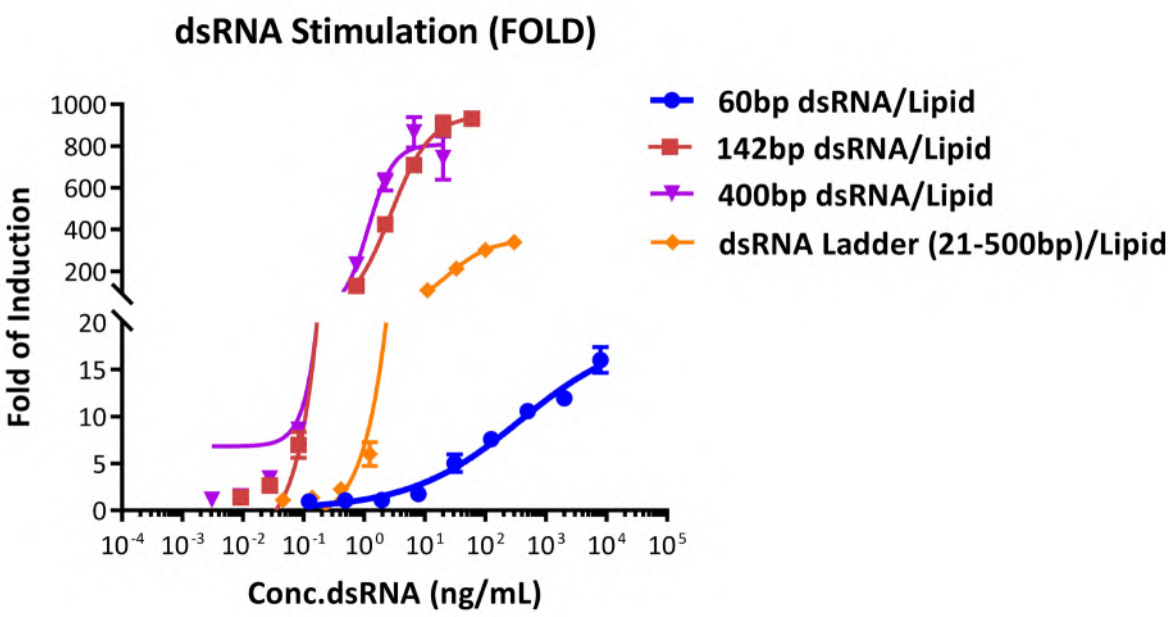
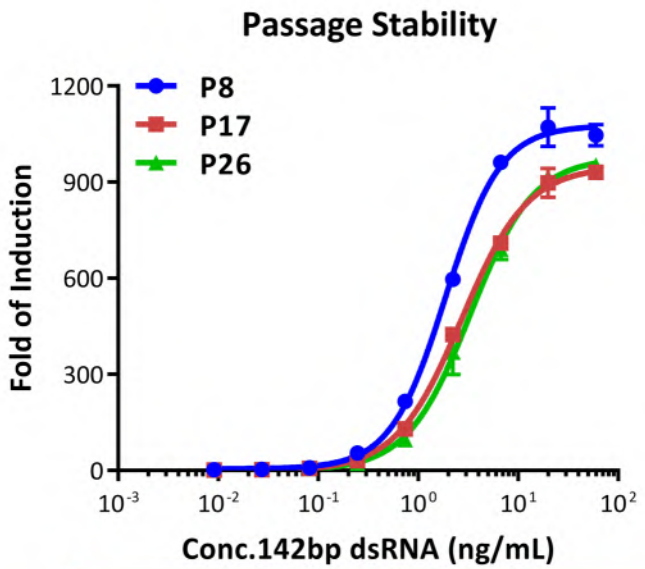



**Fig4. Bioactivity Detection of dsRNA with different lengths (FOLD).** The Human TLR3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Lipofectamine™ RNAiMAX-complexed dsRNA samples with different lengths (60, 142, 400 bp) and a dsRNA ladder (21–500 bp). The induction fold exceeded 5 at approximately 32 ng/mL for 60 bp dsRNA, 0.09 ng/mL for 142 bp dsRNA, 0.09 ng/mL for 400 bp dsRNA, and 1.3 ng/mL for a dsRNA ladder (21–500 bp).

• ***Passage Stability***




| | P8 | P17 | P26 |
|---|---|---|---|
| EC50 (ng/mL) | 1.88 | 2.73 | 3.32 |
| Max Fold | 1071.93 | 930.78 | 952.27 |

**Fig5. Passage stability analysis by Signaling Bioassay.** The continuously growing Human TLR3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of Lipofectamine™ RNAiMAX-complexed 142bp dsRNA. The 142bp dsRNA stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 8-26.

# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

## • *Related Products*

| Products | Cat.No. |
|---|---|
| Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell | CHEK-ATF133 |
| Human TSLP R (Luc) HEK293 Reporter Cell | CHEK-ATF045 |
| STAT3 (Luc) HEK293 Reporter Cell | CHEK-ATF047 |
| HEK293/Human CD40 Ligand / TNFSF5 Stable Cell Line | CHEK-ATP041 |
| HEK293/Human OX40 / TNFRSF4 / CD134 Stable Cell Line | CHEK-ATP053 |
| HEK293/Human OX40 Ligand / TNFSF4 Stable Cell Line | CHEK-ATP054 |
| Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell | CHEK-ATF074 |
| HEK293/FcRn (FCGRT & B2M) Cell Line | CHEK-ATP079 |
| Human IL-21 R (Luc) HEK293 Reporter Cell | CHEK-ATF051 |
| Human IL-11 R alpha (Luc) HEK293 Reporter Cell | CHEK-ATF052 |
| Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell | CHEK-ATF075 |
| CHO/Human TSHR Stable Cell Line | SCCHO-ATP085 |
| HEK293/Human TSHR Stable Cell Line | CHEK-ATP086 |
| Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell | CHEK-ATF094 |
| Human IL-10 R alpha/IL-10 R beta (Luc) HEK293 Reporter Cell | CHEK-ATF095 |
| Human CD40 (Luc) HEK293 Reporter Cell | CHEK-ATF097 |
| Human IL-7 R alpha/CD132 (Luc) HEK293 Reporter Cell | CHEK-ATF099 |
| NIH-3T3/Human IGF-1 R Stable Cell Line Development Service | CNIH-ATP102 |
| Human HVEM (Luc) HEK293 Reporter Cell | CHEK-ATF105 |
| Human BTLA (Luc) Jurkat Reporter Cell | SCJUR-STF106 |
| Human IGF-1 R (Luc) HEK293 Reporter Cell | CHEK-ATF107 |
| Human GLP-2R (Luc) HEK293 Reporter Cell | CHEK-ATF128 |
| Human RANK (Luc) HEK293 Reporter Cell | CHEK-ATF129 |
| HEK293/FcRn (FCGRT & B2M), GFP Tag Stable Cell Line | CHEK-ATP132 |
| Human OX40 (Luc) HEK293 Reporter Cell | CHEK-ATF135 |
| Human IL-2 R beta/IL-2 R gamma (Luc) HEK293 Reporter Cell | CHEK-ATF136 |
| HEK293/Human TL1A Stable Cell Line | CHEK-ATP142 |
| Human IL-23 R/IL-12 R beta 1(Luc) HEK293 Reporter Cell | CHEK-ATF166 |
| Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell | CHEK-ATF167 |

# Human TLR3 (Luc) HEK293 Reporter Cell Data Sheet

## • *Related Products*

| Products | Cat.No. |
|---|---|
| Human DR3 (TL1A receptor) (Luc) Jurkat Reporter Cell | SCJUR-STF178 |
| Human TSHR (Luc) HEK293 Reporter Cell | CHEK-ATF187 |
| CHO/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line | SCCHO-ATP193 |
| Human PTH1R (Luc) HEK293 Reporter Cell | CHEK-ATF194 |
| MDCK/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line Development Service | SCMDC-ATP196 |
| Human TACI (Luc) HEK293 Reporter Cell | CHEK-ATF197 |
| HEK293/Membrane-Bound Human TL1A Stable Cell Line | CHEK-ATF198 |
| Human IL-2 R alpha & IL-2 R beta & IL-2 R gamma (Luc) HEK293 Reporter Cell | CHEK-ATF201 |
| Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell | CHEK-ATF202 |
| Raji/Membrane-Bound Human TL1A Stable Cell Line | SCRAJ-STT204 |
| HEK293/Human MRGPRX2 Stable Cell Line | CHEK-ATP214 |
| CHO/Human MRGPRX2 Stable Cell Line | SCCHO-ATP215 |
| Human TPO R (Luc) HEK293 Reporter Cell | CHEK-ATF226 |