

Human THRA (Luc) HEK293 Reporter Cell Data Sheet

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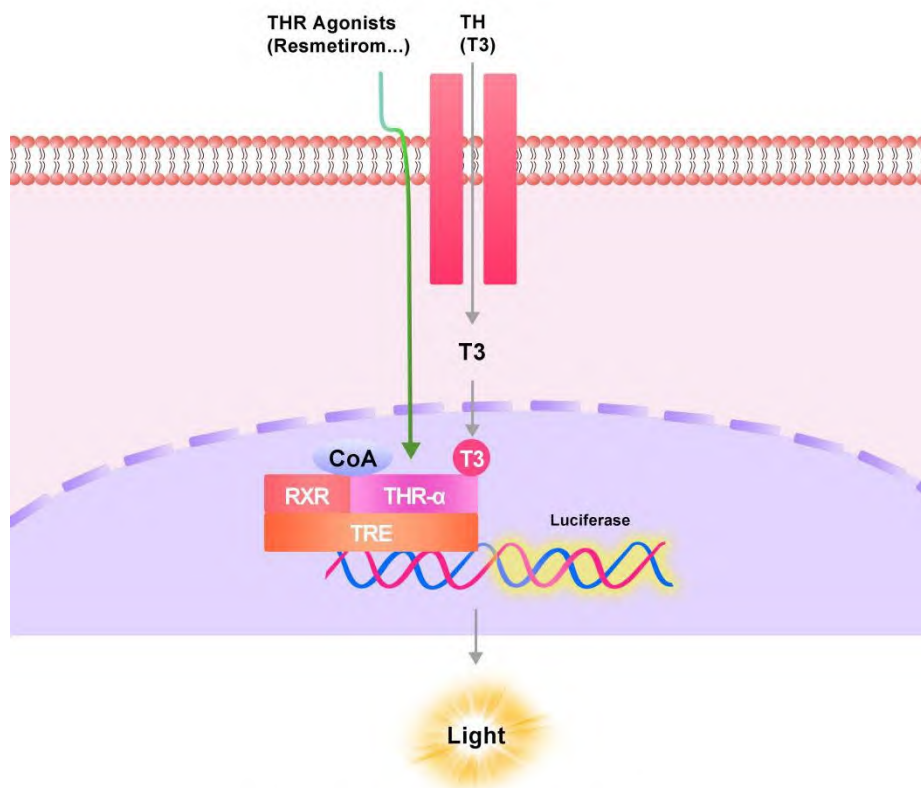
Catalog No.	Size
CHEK-ATF180	2 × (1 vial contains ~5×10 ⁶ cells)

• Description

The Human THRA (Luc) HEK293 Reporter Cell was engineered to not only express thyroid hormone response element (TRE), but also express the receptor full length human THRA (Uniprot: P10827-2), which can drive luciferase expressing systems by thyroid hormone (TH) or thyroid hormone receptor (THR) agonists stimulation. In the absence of TH or THR agonists, the THRA receptor is not activated and luminescence signal is low. In the presence of TH or THR agonists, the THRA pathway-activated luminescence can be detected in a dose-dependent manner.

• Application

- Screen for THR-β-selective agonists



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• Cell Line Profile

Cell line	Human THRA (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) in China, 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)

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• *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°C with 5% CO₂ 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°C with 5% CO₂ 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 THRA (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 5×10^6 to 1×10^7 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.

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• Signaling Bioassay

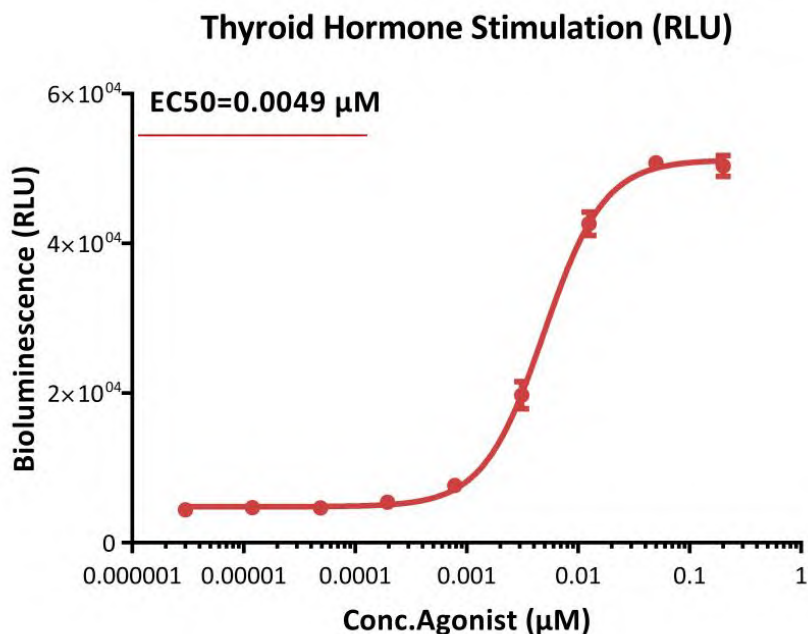


Fig1. Response to thyroid hormone (RLU). This reporter cell was incubated with serial dilutions of Liothyronine (a dual THR- α and THR- β agonist). The EC₅₀ of Liothyronine was approximately 0.0049 μ M.

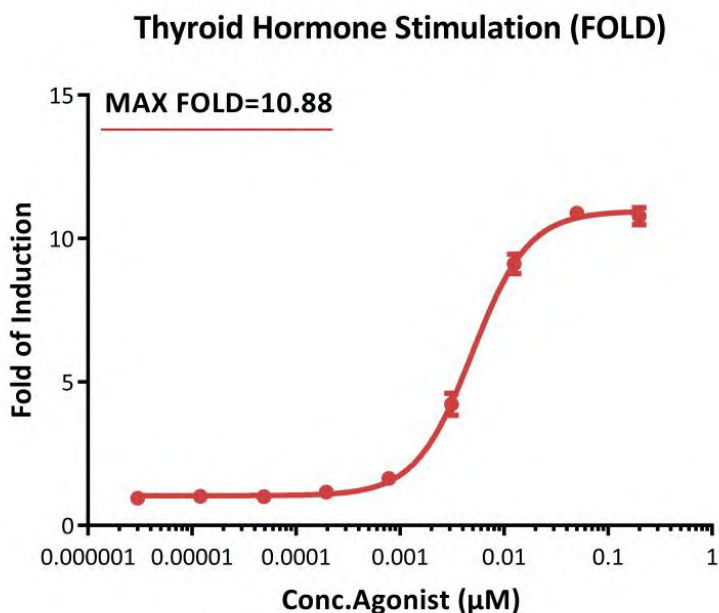


Fig2. Response to thyroid hormone (FOLD). This reporter cell was incubated with serial dilutions of Liothyronine (a dual THR- α and THR- β agonist). The max induction fold was approximately 10.88.

Human THRA (Luc) HEK293 Reporter Cell Data Sheet

• Application

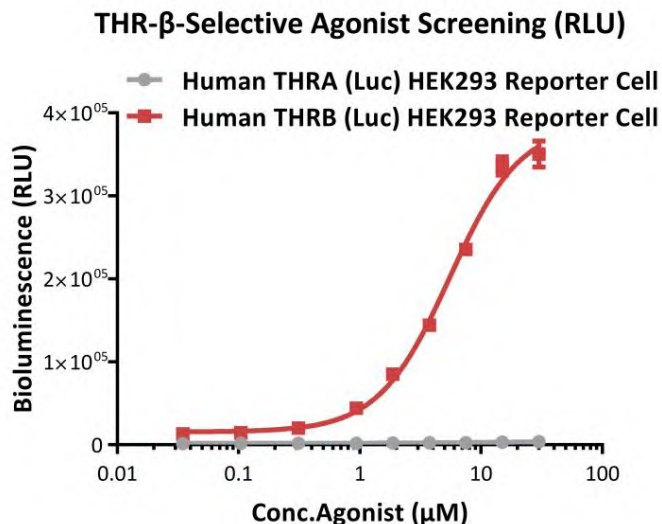


Fig3. Bioactivity analysis of THR-β-selective agonist (RLU). The Human THRA (Luc) HEK293 Reporter Cell and Human THRB (Luc) HEK293 Reporter Cell (Cat. No. CHEK-ATF181) were incubated with serial dilutions of Resmetirom (a THR-β-selective agonist), respectively. The EC₅₀ of Resmetirom determined on Human THRB (Luc) HEK293 Reporter Cell was approximately 5.5 μM.

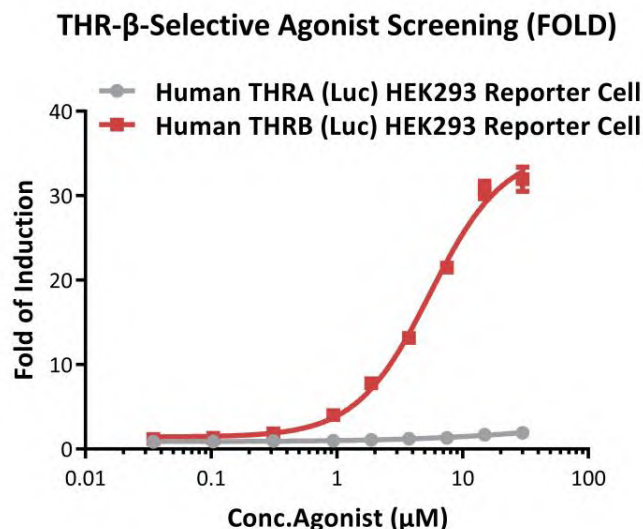


Fig4. Bioactivity analysis of THR-β-selective agonist (FOLD). The Human THRA (Luc) HEK293 Reporter Cell and Human THRB (Luc) HEK293 Reporter Cell (Cat. No. CHEK-ATF181) were incubated with serial dilutions of Resmetirom (a THR-β-selective agonist), respectively. The max induction fold of Resmetirom determined on Human THRB (Luc) HEK293 Reporter Cell was approximately 31.96, and on Human THRA (Luc) HEK293 Reporter Cell was approximately 1.92, which exhibited higher potency and selectivity for THR-β over THR-α.

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• *Related Products*

<u>Products</u>	<u>Cat.No.</u>
Human GLP-1R (Luc) HEK293 Reporter Cell	CHEK-ATF096
Human GCGR (Luc) HEK293 Reporter Cell	CHEK-ATF103
Human GIPR (Luc) HEK293 Reporter Cell	CHEK-ATF104
HEK293/Human GLP-1R Stable Cell Line (High Expression)	CHEK-ATP160
HEK293/Human GLP-1R Stable Cell Line (Medium Expression)	CHEK-ATP161
HEK293/Human GLP-1R Stable Cell Line (Low Expression)	CHEK-ATP162
Human FGF-21 (Luc) HEK293 Reporter Cell	CHEK-ATF163
Human Activin RII (Luc) HEK293 Reporter Cell	CHEK-ATF164
HEK293/Human GPR75 Stable Cell Line	CHEK-ATP174
Human THRB (Luc) HEK293 Reporter Cell	CHEK-ATF181
HEK293/Human GLP-1R&GIPR Stable Cell Line	CHEK-ATP205
HEK293/Human GIPR Stable Cell Line (High Expression)	CHEK-ATP206
HEK293/Human GIPR Stable Cell Line (Medium Expression)	CHEK-ATP207
HEK293/Human GIPR Stable Cell Line (Low Expression)	CHEK-ATP208
HEK293/Human GCGR Stable Cell Line (High Expression)	CHEK-ATP209
HEK293/Human GCGR Stable Cell Line (Medium Expression)	CHEK-ATP210
HEK293/Human GCGR Stable Cell Line (Low Expression)	CHEK-ATP211
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human LDL R Stable Cell Line	CHEK-ATP158