Human THRA (Luc) HEK293 Reporter Cell Data Sheet

Human THRA (Luc) HEK293 Reporter Cell

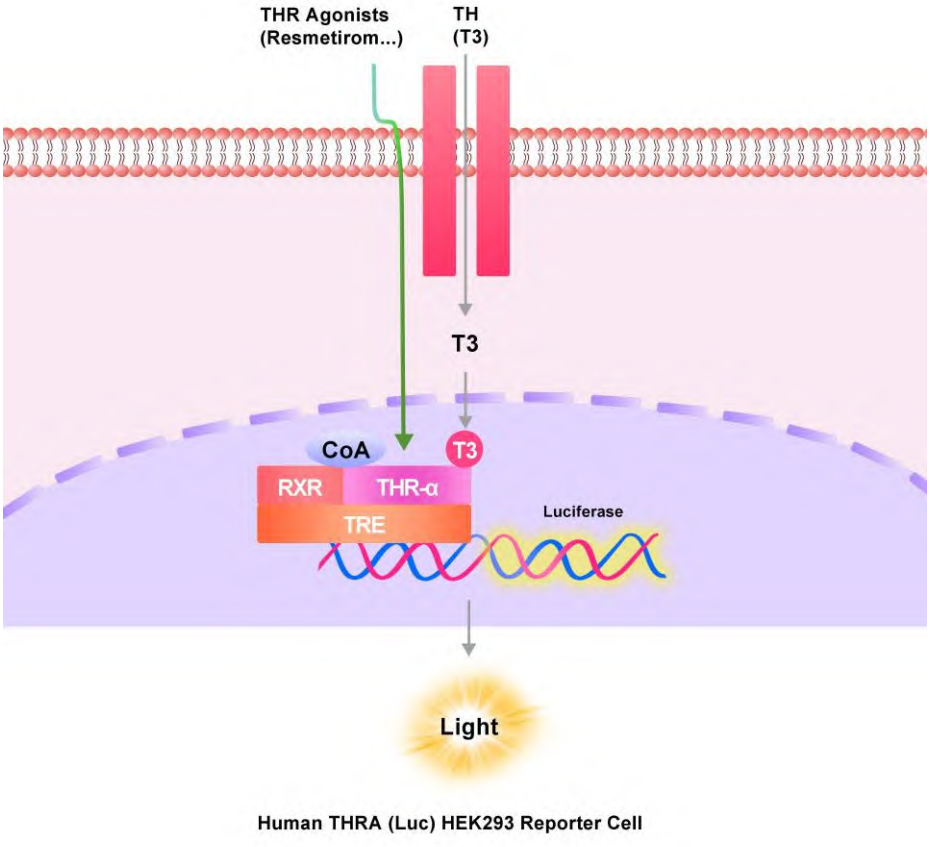
Catalog No.	Size
CHEK-ATF180	2 × (1 vial contains ~5×10^6 cells)

• *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 (Gene ID: 7067), 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 (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 (Gibco, Cat. No. 11965-092)
- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)
- 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 (20 μg/mL) , 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-Ⅱ)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

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• *Recovery*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium and spin at approximately 1000 rpm for 5 minutes.
4. Resuspend cell pellet with 5 mL complete growth medium and transfer the cell suspension into 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. Remove and discard culture medium.
2. Wash the cells once with sterile PBS.
3. Add 2 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached.
4. Add 6.0 to 8.0 mL of culture medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessel.
6. Incubate at 37℃ with 5% CO_2 incubator.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:7 is recommended.

Medium Renewal: Every 2 to 3 days.

Note: After recovery for 1-2 generations with the complete growth medium not containing the selection marker, if the cell state is well, changing to the culture medium containing the selection marker.

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• *Cryopreservation*

1. Remove and discard spent medium.
2. Detach cells from the cell culture flasks with 0.25% trypsin.
3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
4. Resuspend the cell pellets with complete growth medium and count viable cells.
5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transferring to liquid nitrogen storage.

• *Storage*

- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

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- ***Signaling Bioassay***

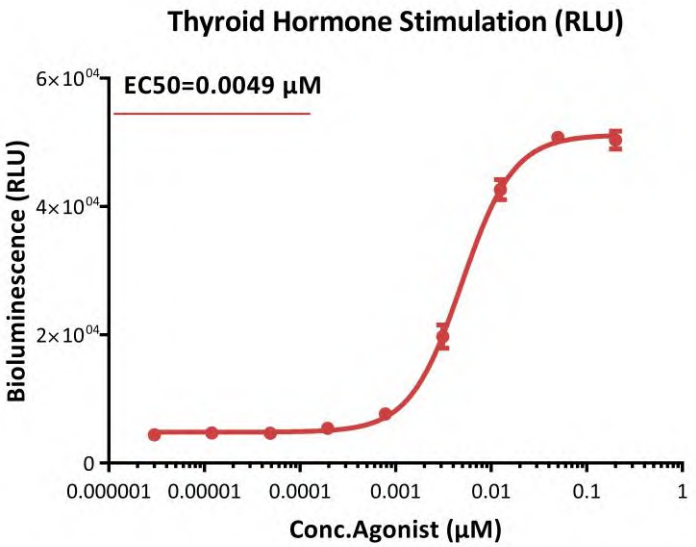

Fig1. Response to thyroid hormone (RLU). This reporter cell was incubated with serial dilutions of Liothyronine (a dual THR-α and THR-β agonist). The EC50 of Liothyronine was approximately 0.0049 μM.

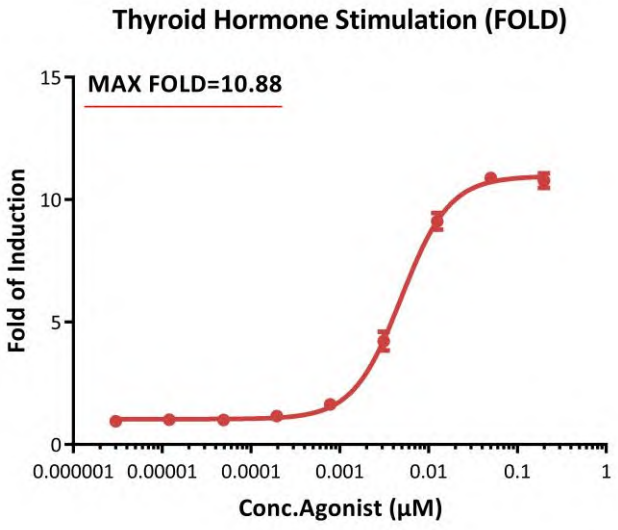

Fig2. Response to thyroid hormone (FOLD). This reporter cell was incubated with serial dilutions of Liothyronine (a dual THR-α and THR-β agonist). The max induction fold was approximately 10.88.

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- ***Application***

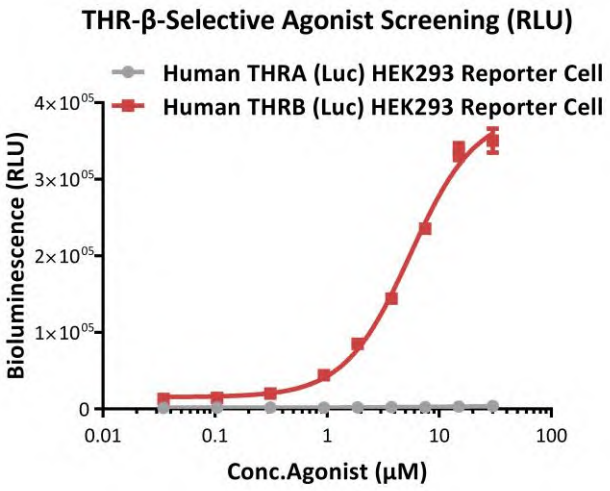

Fig3. Bioactivity analysis of THR-β-selective agonist (RLU). The Human THRA (Luc) HEK293 Reporter Cell and Human THRB (Luc) HEK293 Reporter Cell (Cat. No. CHEK-ATF181) were incubated with serial dilutions of Resmetirom (a THR-β-selective agonist), respectively. The EC50 of Resmetirom determined on Human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

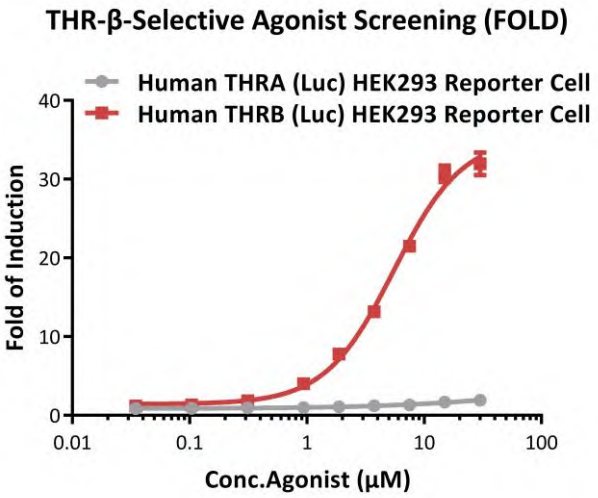

Fig4. Bioactivity analysis of THR-β-selective agonist (FOLD). The Human THRA (Luc) HEK293 Reporter Cell and Human THRB (Luc) HEK293 Reporter Cell (Cat. No. CHEK-ATF181) were incubated with serial dilutions of Resmetirom (a THR-β-selective agonist), respectively. The max induction fold of Resmetirom determined 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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• *License Disclosure*

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• *Related Products*

Products	**Cat. No.**
Human THRB (Luc) HEK293 Reporter Cell	CHEK-ATF181