Human TSHR (Luc) HEK293 Reporter Cell Data Sheet

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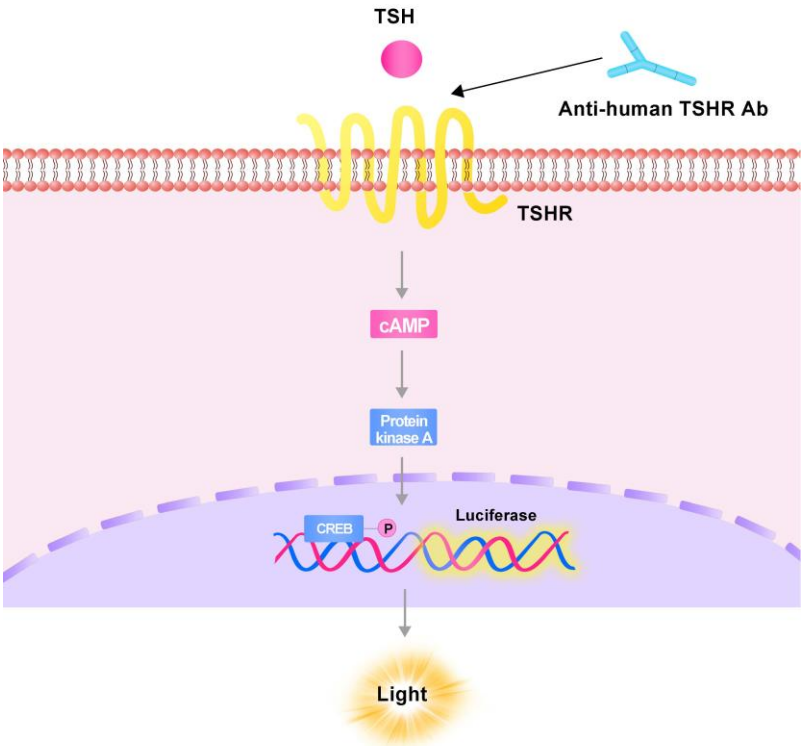
Catalog No.	Size
CHEK-ATF187	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human TSHR (Luc) HEK293 Reporter Cell was engineered to not only express CREB signaling response element, but also express the receptor full length human TSHR (Gene ID: 7253). When stimulated with human TSH protein or TSHR agonist antibody, receptor-mediated signaling can drive CREB-mediated luminescence. Neutralization of biological effect of human TSH protein by corresponding antibody results in a decrease in luminescence.

• *Application*

- Screen for anti-human TSHR agonist antibody.
- Screen for neutralizing antibodies blocking the stimulation of human TSH protein.



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• *Cell Line Profile*

Cell line	Human TSHR (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℃ 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°C 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°C with 5% CO_2 incubator.

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 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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- ***Receptor Assay***

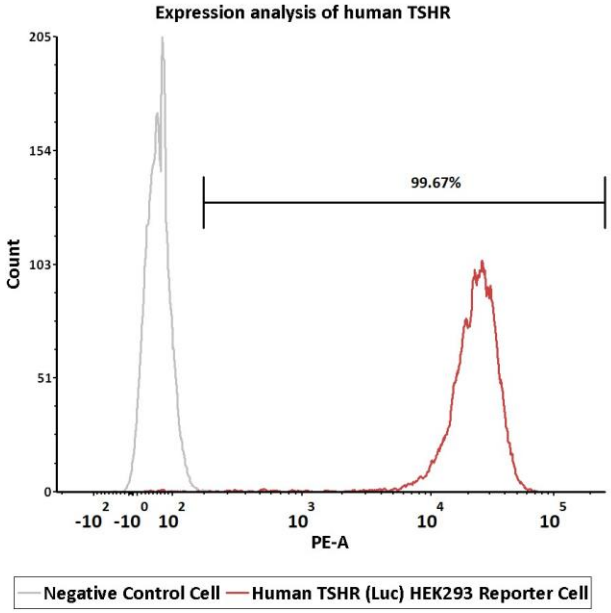

Fig1. Expression analysis of human TSHR on Human TSHR (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human TSHR (Luc) HEK293 Reporter Cell or negative control cell using anti-human TSHR agonistic antibody followed by staining with PE anti-human IgG Fc antibody.

- ***Signaling Bioassay***

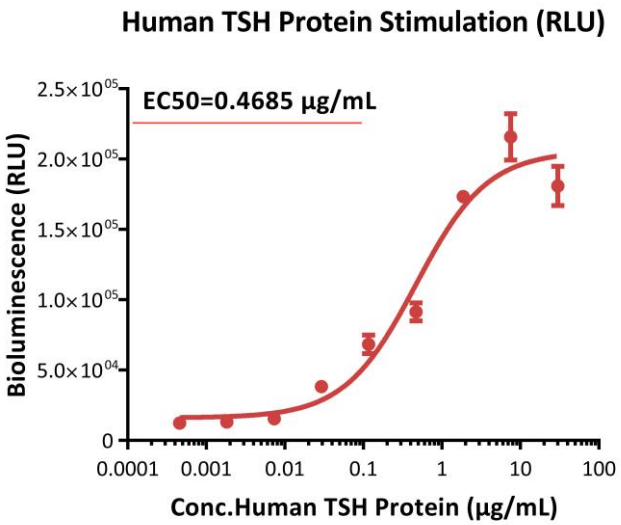

Fig2. Response to human TSH protein (RLU). The Human TSHR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TSH alpha/beta Heterodimer protein (Cat.No.TSR-H52W8). The EC50 was approximately 0.4685 μg/mL.

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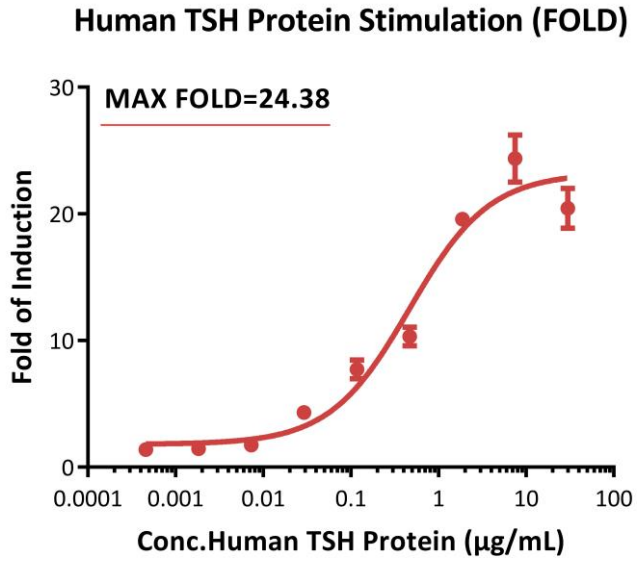

Fig3. Response to human TSH protein (FOLD). The Human TSHR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TSH alpha/beta Heterodimer protein (Cat.No.TSR-H52W8). The max induction fold was approximately 24.38.

- ***Application***

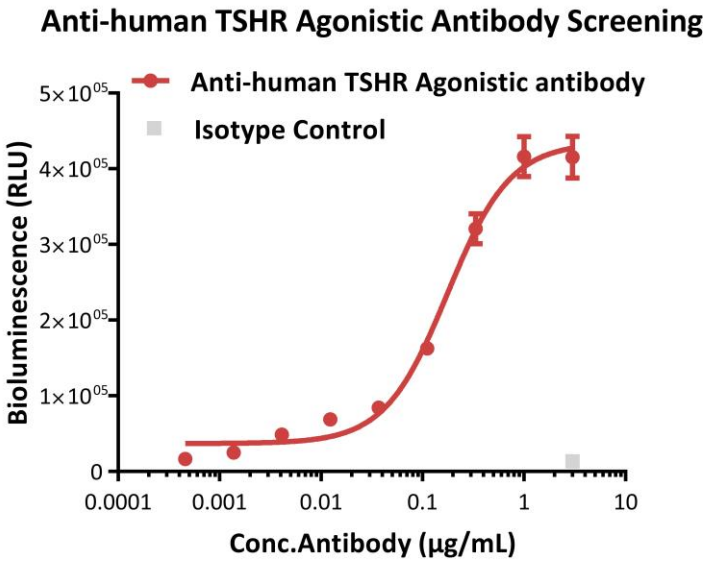

Fig4. Agonistic activity analysis of anti-human TSHR antibody. The Human TSHR (Luc) HEK293 Reporter Cell was incubated with serial dilutions of anti-human TSHR agonistic antibody. The EC50 of anti-human TSHR agonistic antibody was approximately 0.1767 μg/mL with the max induction fold 30.53.

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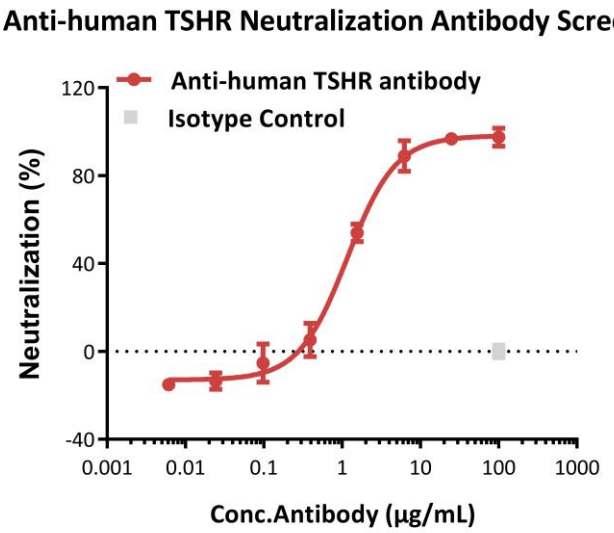

Fig5. Inhibition of human TSH protein-induced reporter activity by anti-human TSHR neutralizing antibody. The Human TSHR (Luc) HEK293 Reporter Cell was incubated with serial dilutions of antibodies in the presence of human TSH alpha/beta Heterodimer protein (Cat.No.TSR-H52W8) with a final concentration of 0.2 μg/mL. The EC50 of anti-human TSHR neutralizing antibody is approximately 1.167 μg/mL.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Human TSH alpha/beta Heterodimer Protein, His Tag	TSR-H52W8
HEK293/Human TSHR Stable Cell Line	CHEK-ATP086
CHO/Human TSHR Stable Cell Line Development Service	SCCHO-ATP085