Human TSHR (Luc) HEK293 Reporter Cell Data Sheet

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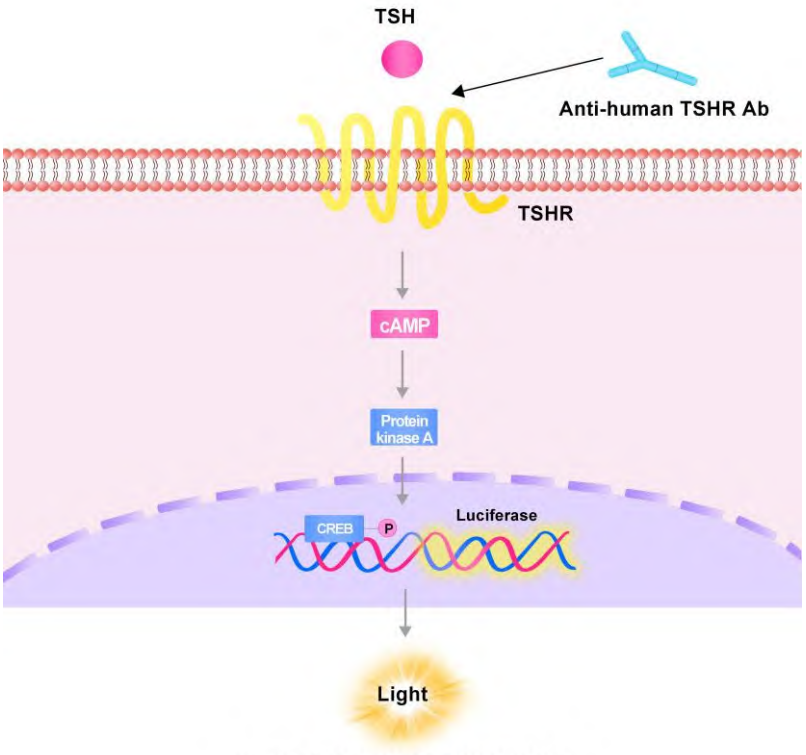
Catalog No.	Size
CHEK-ATF187	2 × (1 vial contains ~5×10^6 cells)

• *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 (Uniprot: P16473-1). 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 B (20 μg/mL)
Incubation	37℃ 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% 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°C 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: 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.

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• *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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- ***Receptor Assay***

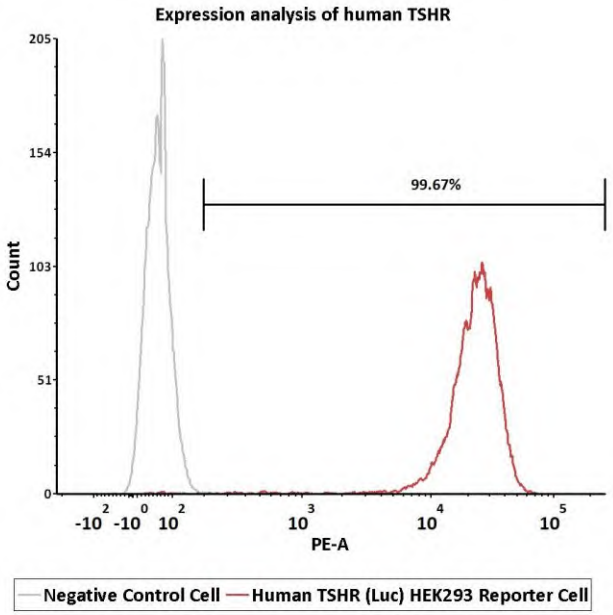

Fig1. Expression analysis of human TSHR on Human TSHR (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human TSHR (Luc) HEK293 Reporter Cell or negative control cell using anti-human TSHR agonistic antibody followed by staining with PE anti-human IgG Fc antibody.

- ***Signaling Bioassay***

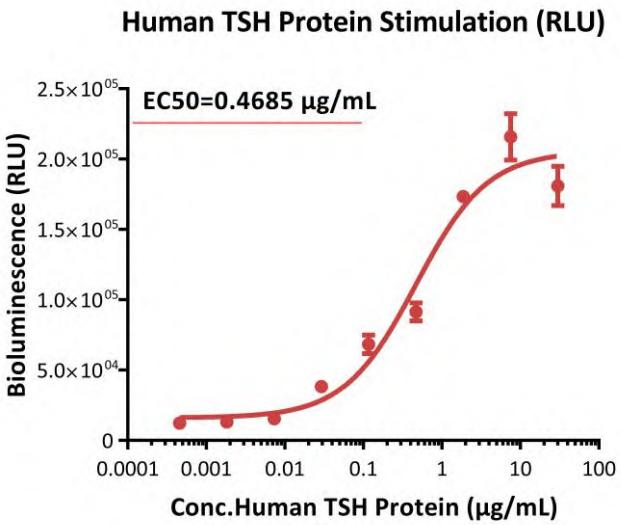

Fig2. Response to human TSH protein (RLU). The Human TSHR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TSH alpha/beta Heterodimer protein (Cat. No. TSR-H52W8). The EC50 was approximately 0.4685 μg/mL.

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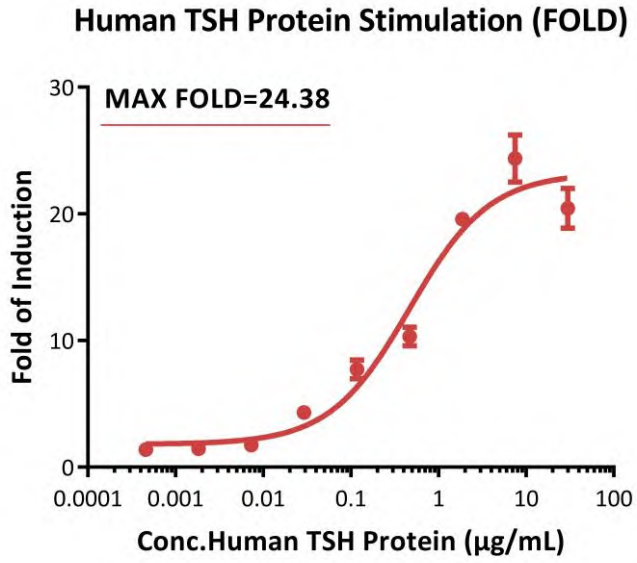

Fig3. Response to human TSH protein (FOLD). The Human TSHR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TSH alpha/beta Heterodimer protein (Cat. No. TSR-H52W8). The max induction fold was approximately 24.38.

- ***Application***

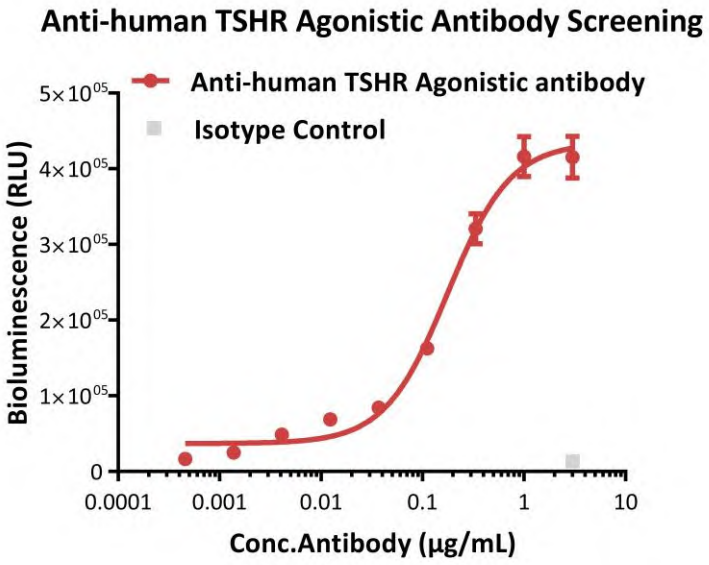

Fig4. Agonistic activity analysis of anti-human TSHR antibody. The Human TSHR (Luc) HEK293 Reporter Cell was incubated with serial dilutions of anti-human TSHR agonistic antibody. The EC50 of anti-human TSHR agonistic antibody was approximately 0.1767 μg/mL with the max induction fold 30.53.

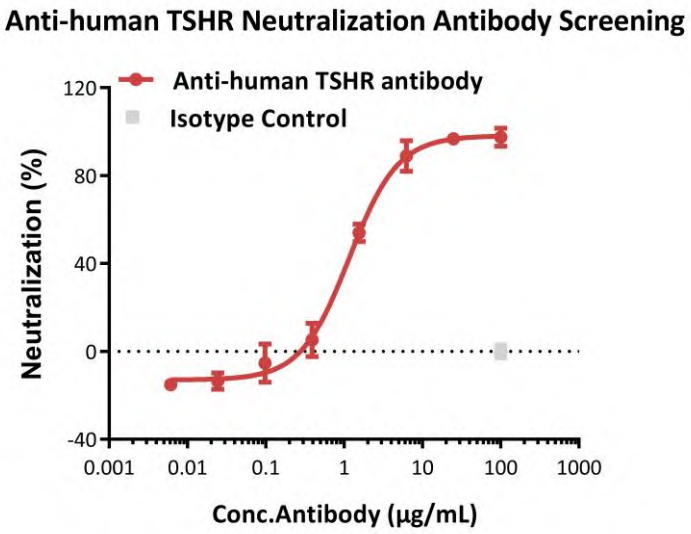

Fig5. Inhibition of human TSH protein-induced reporter activity by anti-human TSHR neutralizing antibody. The Human TSHR (Luc) HEK293 Reporter Cell was incubated with serial dilutions of antibodies in the presence of human TSH alpha/beta Heterodimer protein (Cat. No. TSR-H52W8) with a final concentration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

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• *Related Products*

Products	Cat.No.
Human TSH alpha/beta Heterodimer protein	TSR-H52W8
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
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 IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell	CHEK-ATF133
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

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• *Related Products*

Products	Cat.No.
CHO/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line	SCCHO-ATP193
CHO/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line	CHEK-ATF194
MDCK/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line Development Service	SCMDCATP196
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