Human TSLP R (Luc) HEK293 Reporter Cell Data Sheet

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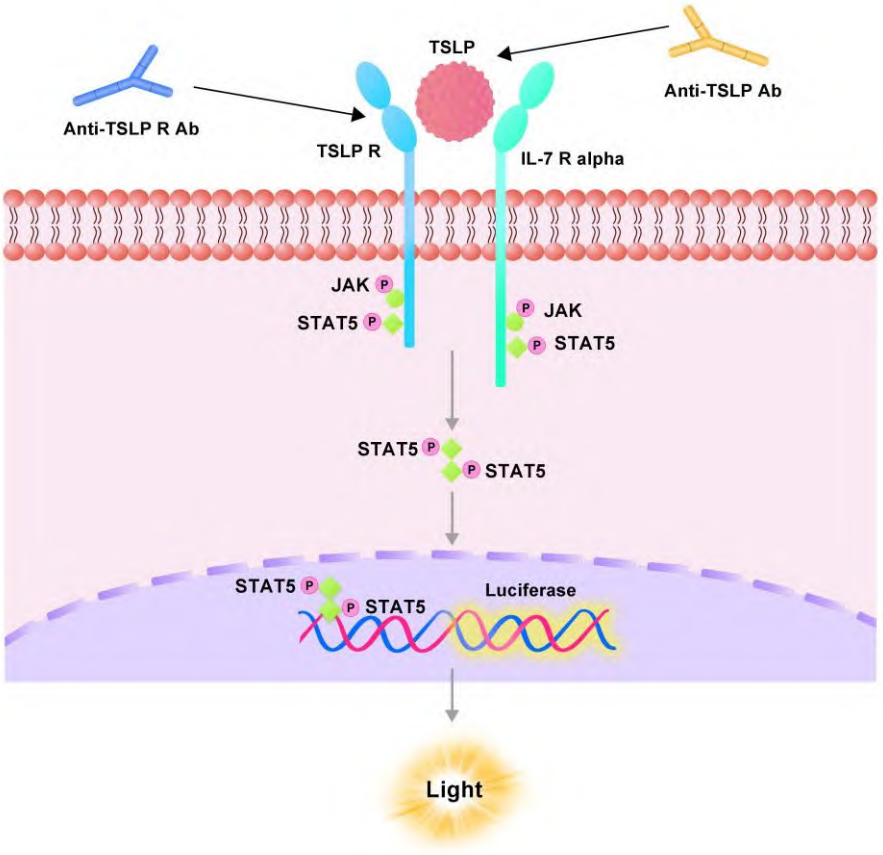
Catalog No.	Size
CHEK-ATF045	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human TSLP R (Luc) HEK293 Reporter Cell was engineered to not only express STAT5 signaling response element, but also express the receptors full length human TSLP R (Uniprot: Q9HC73-1) and IL-7 R alpha (Uniprot: P16871-1). When stimulated with human TSLP protein, the TSLP/TSLP R interaction drives STAT5-mediated luminescence. Inhibition of TSLP binding to TSLP R by either anti-TSLP or anti-TSLP R antibodies results in a decrease in luminescence.

• *Application*

- Screen for anti-human TSLP or anti-human TSLP R neutralizing antibody.



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• *Cell Line Profile*

Cell line	Human TSLP R (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Zeocin (100 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)
- Zeocin (Invitrogen, Cat. No. R25001)

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), Zeocin (100 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)
- CO_2 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***

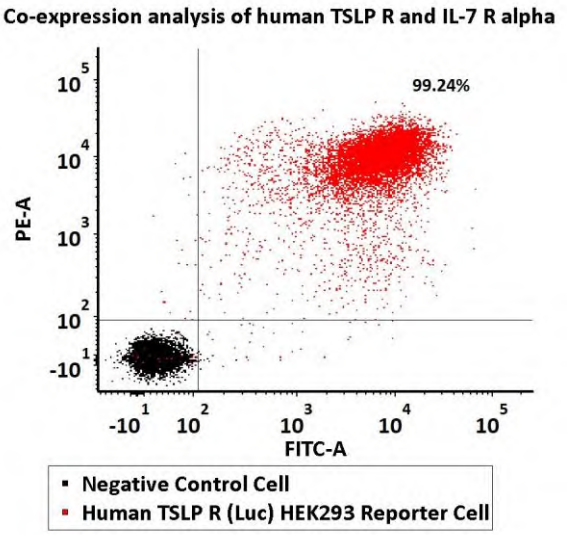

Fig1. Co-expression analysis of human TSLP R and IL-7 R alpha on Human TSLP R (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human TSLP R (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-TSLP R antibody and FITC-labeled anti-IL-7 R alpha antibody.

- ***Signaling Bioassay***

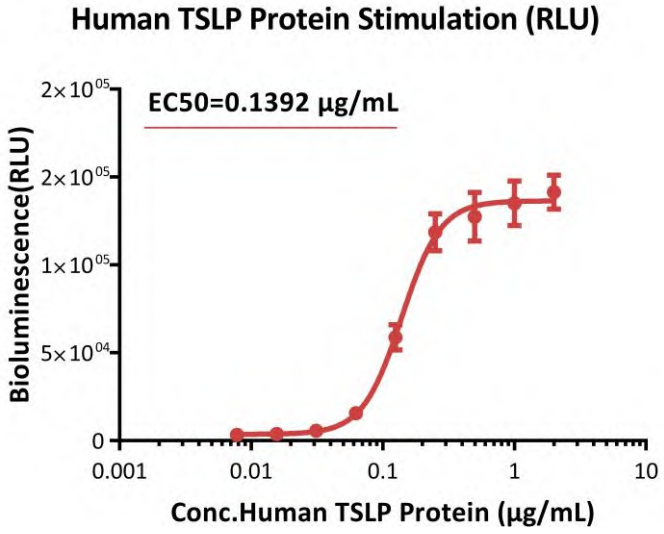

Fig2. Response to human TSLP protein (RLU). The Human TSLP R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TSLP protein (Cat. No. TSP-H52Hb). The EC50 was approximately 0.1392 μg/mL.

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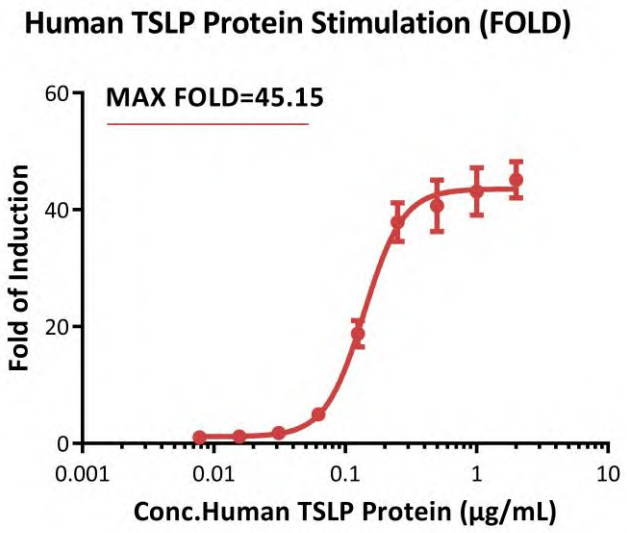

Fig3. Response to human TSLP protein (FOLD). The Human TSLP R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TSLP protein (Cat. No. TSP-H52Hb). The max induction fold was approximately 45.15.

- ***Application***

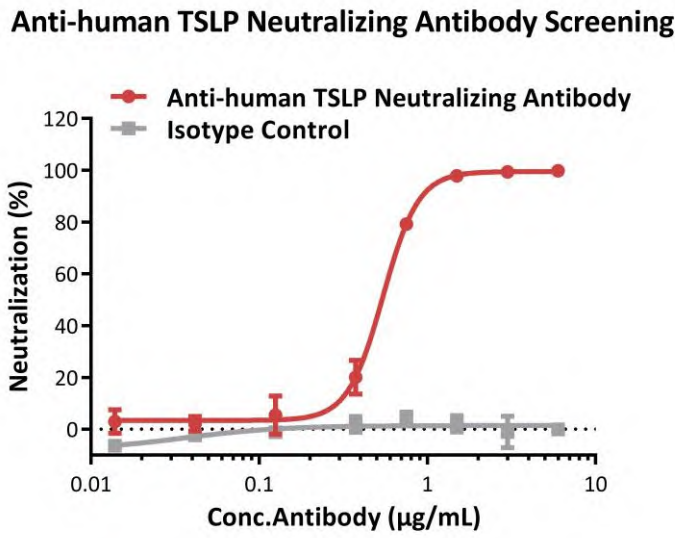

Fig4. Inhibition of human TSLP protein-induced reporter activity by anti-human TSLP neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human TSLP protein (Cat. No. TSP-H52Hb) with a final concentration of 0.3 μg/mL. The EC50 of anti-human TSLP neutralizing antibody is approximately 0.55 μg/mL.

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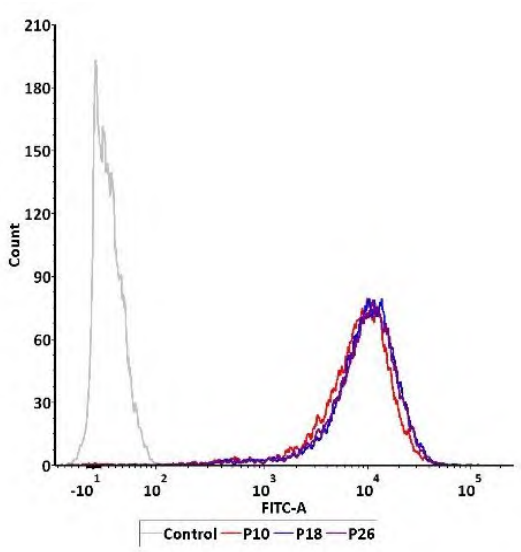
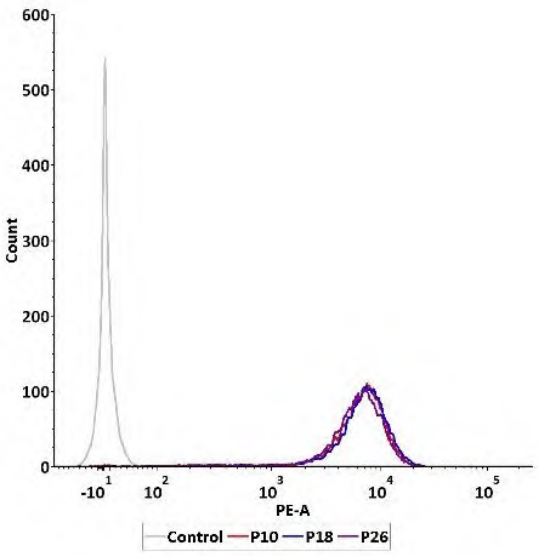
- ***Passage Stability***

A



B



Passage	MFI for IL-7 R alpha (FITC)	MFI for TSLP R (PE)
P10	8267.26	6540.34
P18	9690.88	6838.46
P26	9584.24	6113.65

Fig5. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human TSLP R and IL-7 R alpha on Human TSLP R (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across across passage 10-26. (A) Human IL-7 R alpha expression analysis. (B) Human TSLP R expression analysis.

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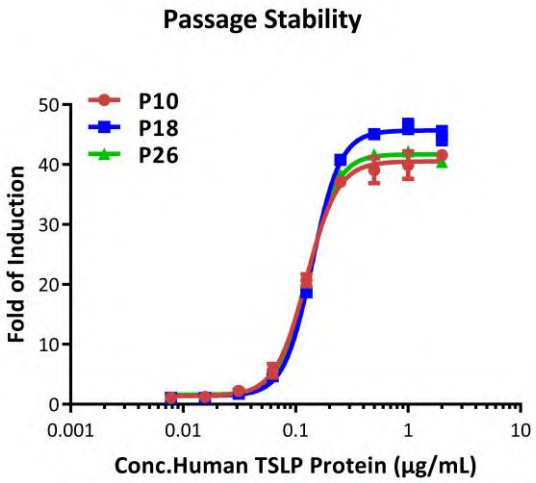

	P10	P18	P26
EC50 (μg/mL)	0.125	0.1408	0.1321
Max Fold	43.36	46.34	42.18

Fig6. Passage stability analysis by Signaling Bioassay. The continuously growing Human TSLP R (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human TSLP protein. Human TSLP protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 10-26.

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• *Related Products*

Products	Cat.No.
Human TSLP Protein	TSP-H52Hb
CHO/Human MRGPRX2 Stable Cell Line	SCCHO-ATP215
CHO/Human LIGHT Stable Cell Line	SCCHO-ATP109
CHO/Human BTLA Stable Cell Line	SCCHO-ATP110
CHO/Human TSHR Stable Cell Line	SCCHO-ATP085
CHO/Human LILRB4 Stable Cell Line	SCCHO-ATP087
Raji/Membrane-Bound Human TL1A Stable Cell Line	SCRAJ-STT204
Human DR3 (TL1A receptor) (Luc) Jurkat Reporter Cell	SCJUR-STF178
Raji/Human HVEM Stable Cell Line	SCRAJ-STF108
Human TSLP R (Luc) HEK293 Reporter Cell	CHEK-ATF045
STAT3 (Luc) HEK293 Reporter Cell	CHEK-ATF047
Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell	CHEK-ATF074
HEK293/Human OX40 / TNFRSF4 / CD134 Stable Cell Line	CHEK-ATP053
HEK293/Human OX40 Ligand / TNFSF4 Stable Cell Line	CHEK-ATP054
HEK293/Human FcRn (FCGRT & B2M) Stable Cell Line	CHEK-ATP079
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
Human IL-21 R/CD132 (Luc) HEK293 Reporter Cell	CHEK-ATF051
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
HEK293/Human LILRB4 Stable Cell Line	CHEK-ATP088
HEK293/Human TL1A Stable Cell Line	CHEK-ATP142
Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell	CHEK-ATF133

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• *Related Products*

Products	Cat.No.
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 HVEM Stable Cell Line	CHEK-ATP147
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
HEK293/Human CD40 Ligand / TNFSF5 Stable Cell Line	CHEK-ATP041
Human TSHR (Luc) HEK293 Reporter Cell	CHEK-ATF187
Human PTH1R (Luc) HEK293 Reporter Cell	CHEK-ATF194
HEK293/Membrane-Bound human TL1A Stable Cell Line	CHEK-ATP198
Human TACI (Luc) HEK293 Reporter Cell	CHEK-ATF197
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
HEK293/Human TSHR Stable Cell Line	CHEK-ATP086