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Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell

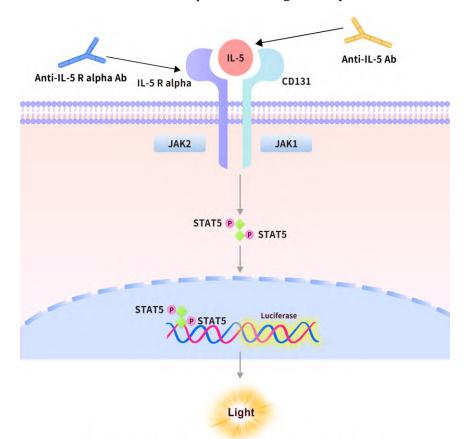
Catalog No.	Size
CHEK-ATF074	$2 \times (1 \text{ vial contains } \sim 5 \times 10^6 \text{ cells})$

• Description

The Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell was engineered to not only express STAT5 signaling response element, but also express the receptors full length human IL-5 R alpha (Uniprot: Q01344-1) and CD131 (Uniprot: P32927-1). When stimulated with human IL-5 protein, the IL-5/ IL-5 R alpha interaction drives STAT5-mediated luminescence. Inhibition of IL-5 binding to IL-5 R alpha by either anti-IL-5 or anti-IL-5 R alpha antibodies results in a decrease in luminescence.

• Application

• Screen for anti-human IL-5 or anti-human IL-5 R alpha neutralizing antibody.



Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell



• Cell Line Profile

Cell line	Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	NA
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)
- 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
- 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)



• 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 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.
- 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.



• 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.



• Receptor Assay

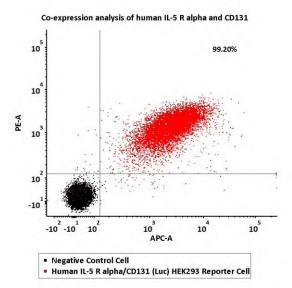


Fig1. Co-expression analysis of human IL-5 R alpha and CD131 on Human IL-5 R alpha/CD131 (Luc) **HEK293 Reporter Cell by FACS.** Cell surface staining was performed on Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-IL-5 R alpha antibody and APC-labeled anti-CD131 antibody.

• Signaling Bioassay

Human IL-5 Protein Stimulation (RLU)

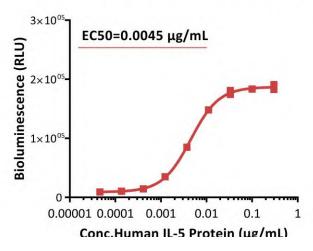


Fig2. Response to human IL-5 protein (RLU). The Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-5 protein (Cat. No. IL5-H52H3). The EC50 was approximately $0.0045~\mu g/mL$.



Human IL-5 Protein Stimulation (FOLD)

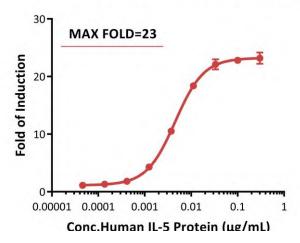


Fig3. Response to human IL-5 protein (Fold). The Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-5 protein (Cat. No. IL5-H52H3). The max induction fold was approximately 23.

Application

Anti-human IL-5 Neutralizing Antibody Screening

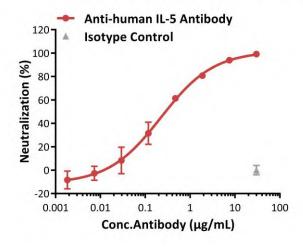


Fig4. Inhibition of human IL-5 protein-induced reporter activity by anti-human IL-5 neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-5 protein (Cat. No. IL5-H52H3) with a final concentration of $0.01\mu g/mL$. The EC50 of anti-human IL-5 neutralizing antibody is approximately $0.22 \mu g/mL$.



• Passage Stability

P23

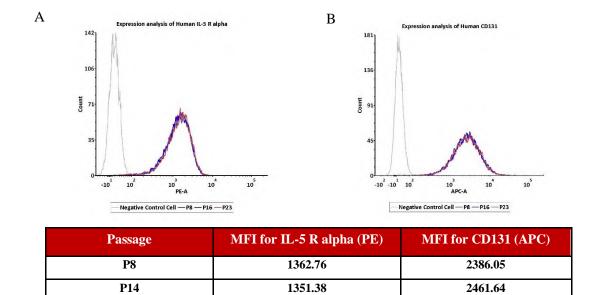


Fig5. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human IL-5 R alpha and CD131 on Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across passage 8-23. (A) Human IL-5 R alpha expression analysis. (B) Human CD131 expression analysis.

1429.69

2421.33



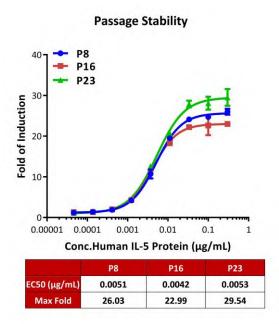


Fig6. Passage stability analysis by Signaling Bioassay. The continuously growing Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-5 protein (Cat. No. IL5-H52H3). Human IL-5 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage



• Related Products

<u>Products</u>	Cat.No.
Human IL-5 protein	IL5-H52H3
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
Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell	CHEK-ATF167



• Related Products

<u>Products</u>	Cat.No.
Human DR3 (TL1A receptor) (Luc) Jurkat Reporter Cell	SCJUR-STF178
Human TSHR (Luc) HEK293 Reporter Cell	CHEK-ATF187
CHO/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line	SCCHO-ATP193
Human PTH1R (Luc) HEK293 Reporter Cell	CHEK-ATF194
MDCK/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line Development Service	SCMDC-ATP196
Human TACI (Luc) HEK293 Reporter Cell	CHEK-ATF197
HEK293/Membrane-Bound Human TL1A Stable Cell Line	CHEK-ATP198
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