

Human IL-1RL1 & IL-1 RAcP (IL-33 receptor) (Luc) HEK293 Reporter Cell Data Sheet

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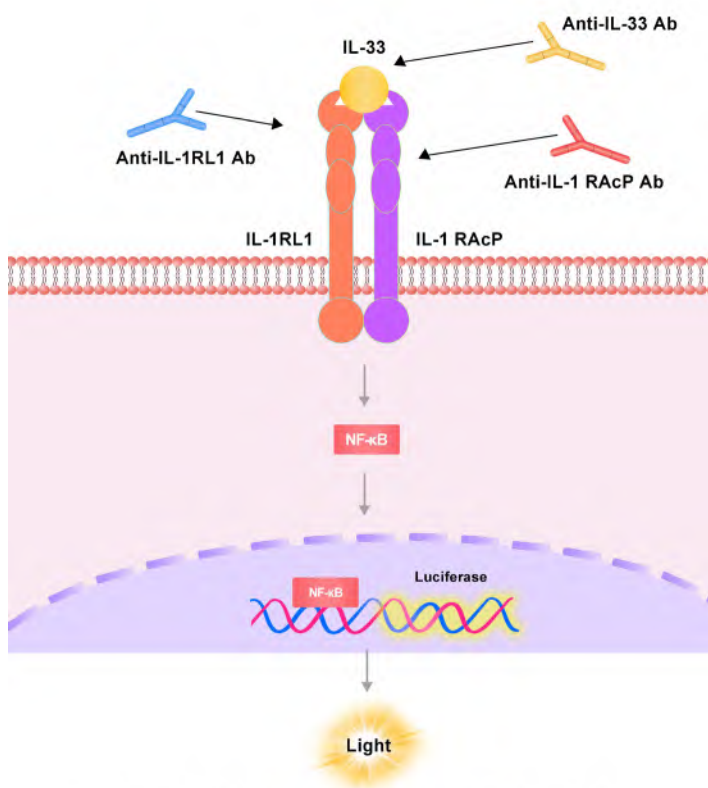
Catalog No.	Size
CHEK-ATF244	2 × (1 vial contains ~5×10 ⁶ cells)

• Description

The Human IL-1RL1 & IL-1 RAcP (IL-33 receptor) (Luc) HEK293 Reporter Cell expressing human IL-1 RAcP was engineered to not only express NF-κB signaling response element, but also express the receptor full length human IL-1RL1 (Uniprot: Q01638-1). When stimulated with human IL-33 protein, receptor-mediated signaling can drive NF-κB-mediated luminescence. Neutralization of biological effect of the ligand-receptor interaction by corresponding antibody results in a decrease in luminescence.

• Application

- Screen for neutralizing antibodies blocking the stimulation of human IL-33 protein



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• Cell Line Profile

Cell line	Human IL-1RL1 & IL-1 RAcP (IL-33 receptor) (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°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)
- 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% 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 **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% 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.

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

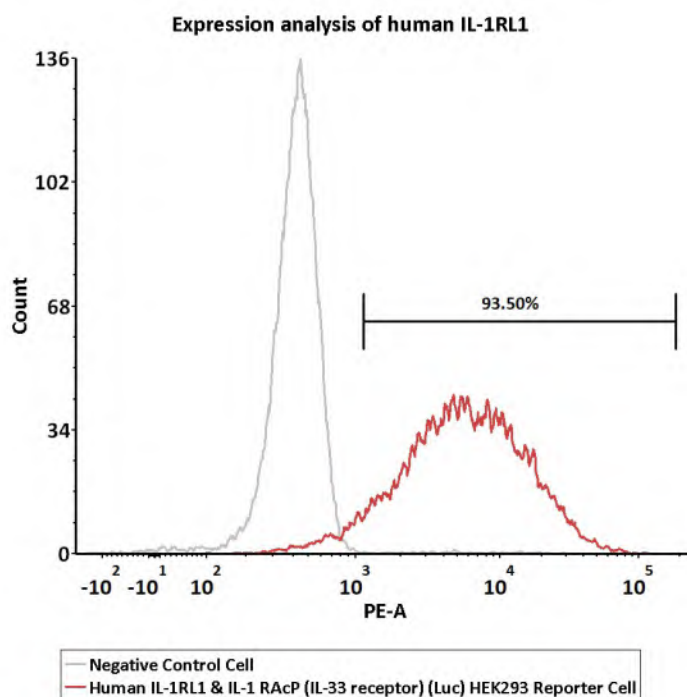


Fig1. Expression analysis of human IL-1RL1 on Human IL-1RL1 & IL-1 RAcP (IL-33 receptor) (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human IL-1RL1 & IL-1 RAcP (IL-33 receptor) (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human IL-1RL1 antibody.

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• Signaling Bioassay

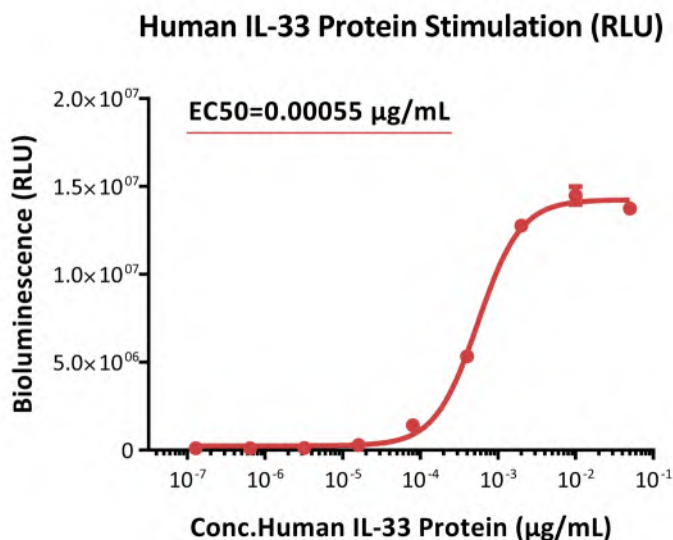


Fig2. Response to human IL-33 protein (RLU). The Human IL-1 R1 & IL-1 RAcP (IL-33 receptor) (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-33 protein (Cat. No. IL3-H52H7). The EC₅₀ was approximately 0.00055 µg/mL.

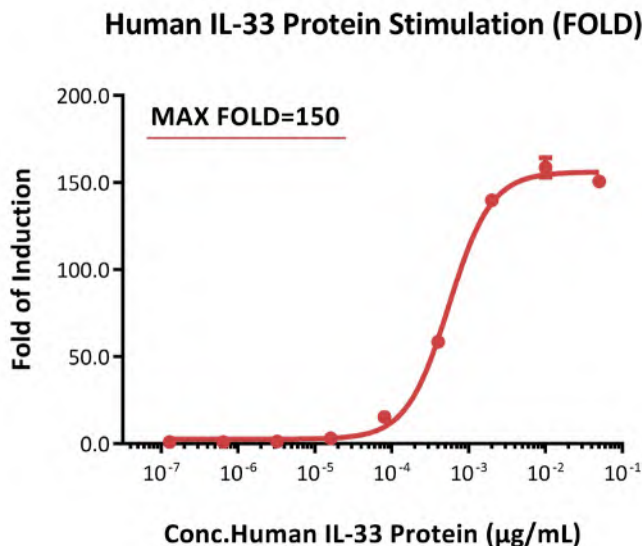


Fig3. Response to human IL-33 protein (FOLD). The Human IL-1 R1 & IL-1 RAcP (IL-33 receptor) (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-33 protein (Cat. No. IL3-H52H7). The max induction fold was approximately 150.

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• Application

Anti-human IL-33 Neutralizing Antibody Screening

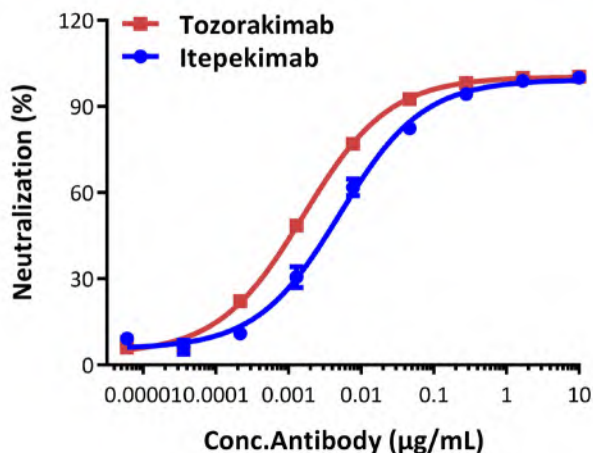


Fig4. Inhibition of human IL-33 protein-induced reporter activity by anti-human IL-33 neutralizing antibody. This reporter cell was incubated with serial dilutions of anti-human IL-33 neutralizing antibodies in the presence of human IL-33 protein (Cat. No. IL3-H52H7) with a final concentration of 0.0001 µg/mL. The EC50 of Tozorakimab is approximately 0.0016 µg/mL, and the EC50 of Itepekimab is approximately 0.0050 µg/mL.

Anti-human IL-1RL1 Neutralizing Antibody Screening

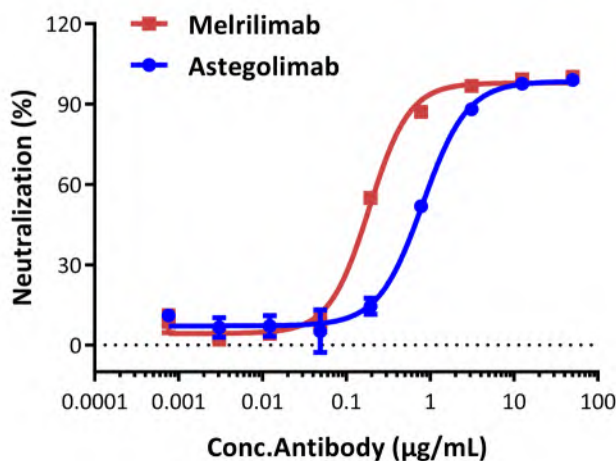


Fig5. Inhibition of human IL-33 protein-induced reporter activity by anti-human IL-1RL1 neutralizing antibody. This reporter cell was incubated with serial dilutions of anti-human IL-1RL1 neutralizing antibodies in the presence of human IL-33 protein (Cat. No. IL3-H52H7) with a final concentration of 0.0001 µg/mL. The EC50 of Melrilimab is approximately 0.1882 µg/mL, and the EC50 of Astegolimab is approximately 0.8173 µg/mL.

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• Related Products

<u>Products</u>	<u>Cat. No.</u>
Human IL-1 alpha / IL-1F1 Protein, premium grade	ILA-H5214
Human IL-1 beta / IL-1F2 Protein, premium grade	ILB-H4110
Human IL-2 R beta/IL-2 R gamma (Luc) HEK293 Reporter Cell	CHEK-ATF136
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
Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell	CHEK-ATF133
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 IL-7 R alpha/CD132 (Luc) HEK293 Reporter Cell	CHEK-ATF099
Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell	CHEK-ATF074
Human IGF-1 R (Luc) HEK293 Reporter Cell	CHEK-ATF107
NIH-3T3/Human IGF-1 R Stable Cell Line Development Service	CNIH-ATP102
Human CD40 (Luc) HEK293 Reporter Cell	CHEK-ATF097
HEK293/Human OX40 / TNFRSF4 / CD134 Stable Cell Line	CHEK-ATP053
HEK293/Human OX40 Ligand / TNFSF4 Stable Cell Line	CHEK-ATP054
Human OX40 (Luc) HEK293 Reporter Cell	CHEK-ATF135
HEK293/Human CD40 Ligand / TNFSF5 Stable Cell Line	CHEK-ATP041
HEK293/Human FcRn (FCGRT & B2M) Stable Cell Line	CHEK-ATP079
Raji/Membrane-Bound human TL1A Stable Cell Line	SCRAJ-STT204
HEK293/Membrane-Bound human TL1A Stable Cell Line	CHEK-ATP198
HEK293/Human TL1A Stable Cell Line	CHEK-ATP142
Human DR3 (TL1A receptor) (Luc) Jurkat Reporter Cell	SCJUR-STF178
HEK293/Human HVEM Stable Cell Line	CHEK-ATP147

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• *Related Products*

<u>Products</u>	<u>Cat. No.</u>
Human TSLP R (Luc) HEK293 Reporter Cell	CHEK-ATF045
STAT3 (Luc) HEK293 Reporter Cell	CHEK-ATF047
Human HVEM (Luc) HEK293 Reporter Cell	CHEK-ATF105
Human BTLA (Luc) Jurkat Reporter Cell	SCJUR-STF106
Raji/Human HVEM Stable Cell Line	SCRAJ-STF108
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
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
HEK293/Human LILRB4 Stable Cell Line	CHEK-ATP088
Human TSHR (Luc) HEK293 Reporter Cell	CHEK-ATF187
Human PTH1R (Luc) HEK293 Reporter Cell	CHEK-ATF194
Human TACI (Luc) HEK293 Reporter Cell	CHEK-ATF197
Human IL-2 R alpha & IL-2 R beta & IL-2 R gamma (Luc) HEK293 Reporter Cell	CHEK-ATF201