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## Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell

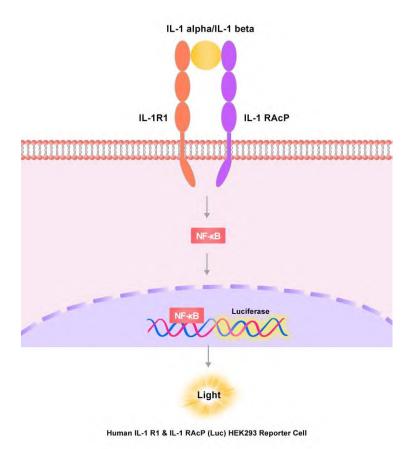
Catalog No.	Size
CHEK-ATF202	$2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$

#### • Description

The Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell was engineered to express NF-κB signaling response element. When stimulated with human IL-1 alpha or IL-1 beta 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-1 alpha or IL-1 beta protein.
- Screen for anti-human IL-1 R1 or IL-1 RAcP neutralizing antibody.



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#### • Cell Line Profile

Cell line	Human IL-
Host Cell	
Property	
Complete Growth Medium	
Selection Marker	
Incubation	
Doubling Time	
Transduction Technique	

Iman IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell HEK293 Adherent DMEM + 10% FBS Puromycin (2 µg/mL) 37°C with 5% CO<sub>2</sub> 22-24 hours 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)
- 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), 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<sub>2</sub> Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)



#### • 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<sub>2</sub> 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<sub>2</sub> 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.

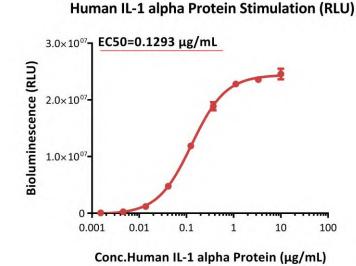


#### • Cryopreservation

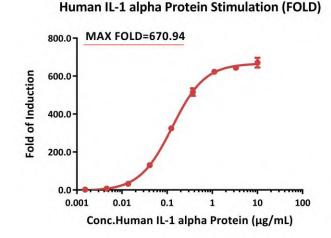
- 1. Remove and discard spent medium.
- 2. Detach cells from the cell culture flasks with 0.25% trypsin.
- 3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
- 4. Resuspend the cell pellets with complete growth medium and count viable cells.
- 5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of  $5 \times 10^6$  to  $1 \times 10^7$  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



#### • Signaling Bioassay



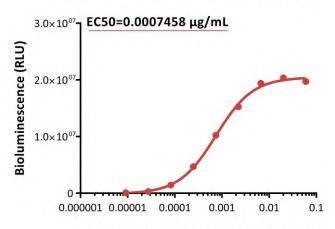
**Fig1. Response to human IL-1 alpha protein (RLU).** The Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-1 alpha protein (Cat. No. ILA-H5214). The EC50 was approximately 0.1293 µg/mL.



**Fig2. Response to human IL-1 alpha protein (FOLD).** The Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-1 alpha protein (Cat. No. ILA-H5214). The max induction fold was approximately 670.94.

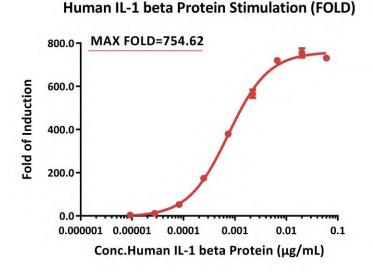


Human IL-1 beta Protein Stimulation (RLU)



Conc.Human IL-1 beta Protein (µg/mL)

**Fig3. Response to human IL-1 beta protein (RLU).** The Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-1 beta protein (Cat. No. ILB-H4110). The EC50 was approximately 0.0007458 μg/mL.



**Fig4. Response to human IL-1 beta protein (FOLD).** The Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-1 beta protein (Cat. No. ILB-H4110). The max induction fold was approximately 754.62.



Anti-human IL-1 RAcP Neutralization Antibody Screening

#### • Application

Anti-human IL-1 RAcP neutralization antibody Isotype Control 0001 0.001 0.01 0.1 1 10 Conc.Antibody (µg/mL)

Fig5. Inhibition of human IL-1 alpha protein-induced reporter activity by anti-human IL-1 RAcP neutralization antibody. The Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell was incubated with serial dilutions of antibodies in the presence of IL-1 alpha protein (Cat. No. ILA-H5214) with a final concentration of 0.02  $\mu$ g/mL. The EC50 of anti-human IL-1 RAcP neutralization antibody is approximately 0.01347  $\mu$ g/mL.

#### Anti-human IL-1 RAcP Neutralization Antibody Screening

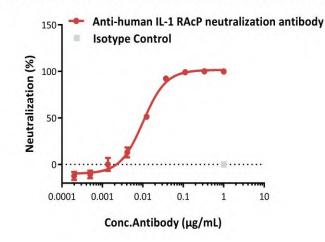


Fig6. Inhibition of human IL-1 beta protein-induced reporter activity by anti-human IL-1 RAcP neutralization antibody. The Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell was incubated with serial dilutions of antibodies in the presence of IL-1 beta protein (Cat. No. ILB-H4110) with a final concentration of 0.0002  $\mu$ g/mL. The EC50 of anti-human IL-1 RAcP neutralization antibody is approximately 0.01014 $\mu$ g/mL.



#### • Related Products

Products	<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 Development Service	SCJUR-STF178
HEK293/Human HVEM Stable Cell Line	CHEK-ATP147



#### • Related Products

Products	<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 Development Service	SCJUR-STF106
Raji/Human HVEM Stable Cell Line Development Service	SCRAJ-STF108
CHO/Human LIGHT Stable Cell Line Development Service	SCCHO-ATP109
CHO/Human BTLA Stable Cell Line Development Service	SCCHO-ATP110
CHO/Human TSHR Stable Cell Line Development Service	SCCHO-ATP085
CHO/Human LILRB4 Stable Cell Line Development Service	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	CHEK-ATF201
Cell	