Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell

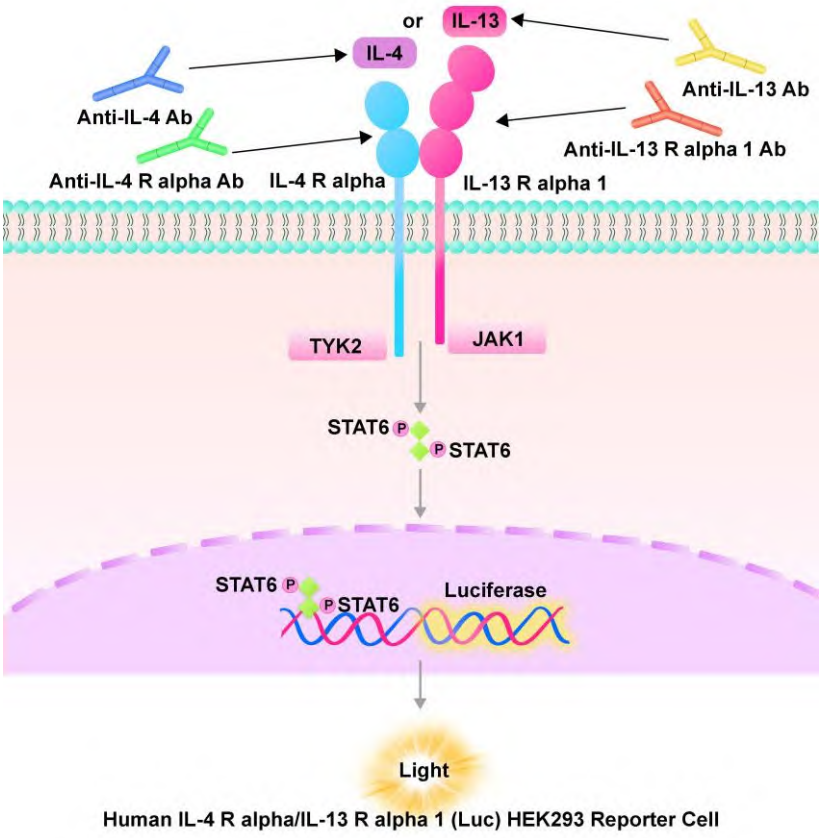
Catalog No.	Size
CHEK-ATF075	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell with endogenous IL-4 R alpha and IL-13 R alpha 1 expression was engineered to express STAT6 signaling response element. When stimulated with human IL-4 or IL-13 protein, receptor-mediated signaling can drive STAT6-mediated luminescence. Neutralization of biological effect of human IL-4 or IL-13 protein by corresponding antibody results in a decrease in luminescence.

• *Application*

- Screen for neutralizing antibodies blocking the stimulation of human IL-4 or IL-13 protein.



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• *Cell Line Profile*

Cell line	Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- DMEM medium (Gibco, Cat. No. 11965-092)
- 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, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-Ⅱ)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 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. Discard the supernatant and 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℃ with 5% CO_2 incubator.

• *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℃ with 5% CO_2 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 5×10^6 to 1×10^7 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

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- ***Signaling Bioassay***

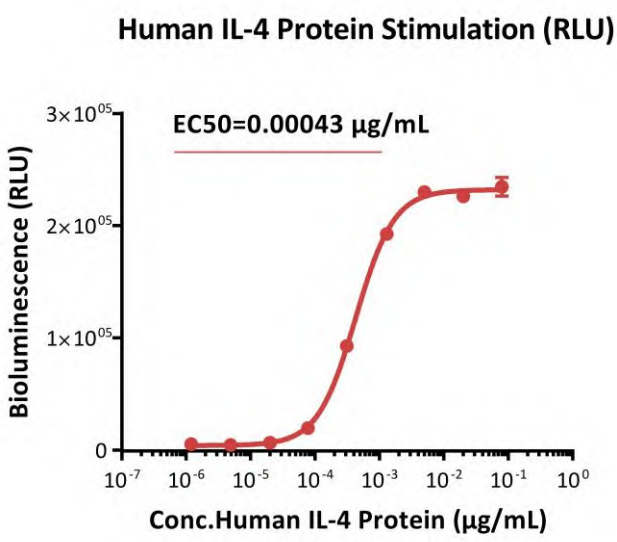

Fig1. Response to human IL-4 protein (RLU). The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-4 protein (Cat. No. IL4-H4218). The EC50 was approximately 0.00043 μg/mL.

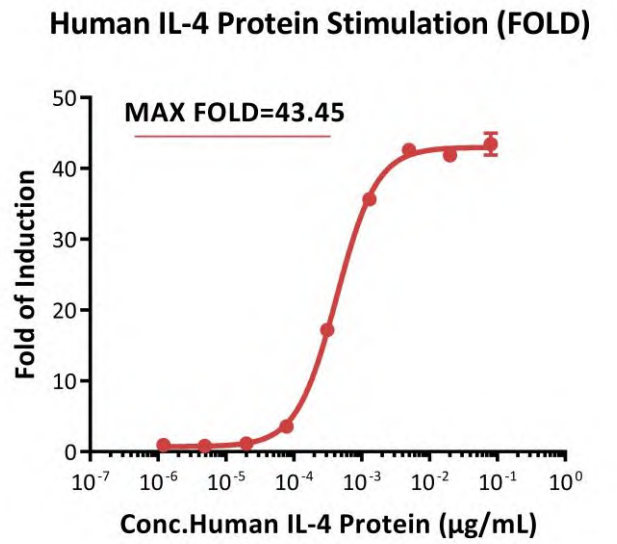

Fig2. Response to human IL-4 protein (FOLD). The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-4 protein (Cat. No. IL4-H4218). The max induction fold was approximately 43.45.

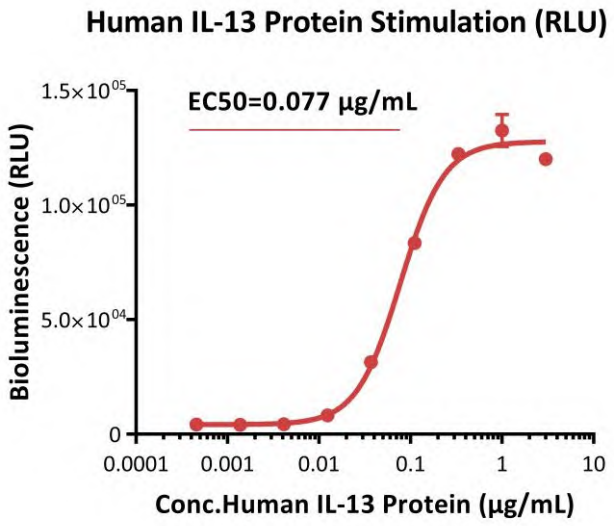

Fig3. Response to human IL-13 protein (RLU). The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-13 protein (Cat. No. IL3-H52H4). The EC50 was approximately 0.077 μg/mL.

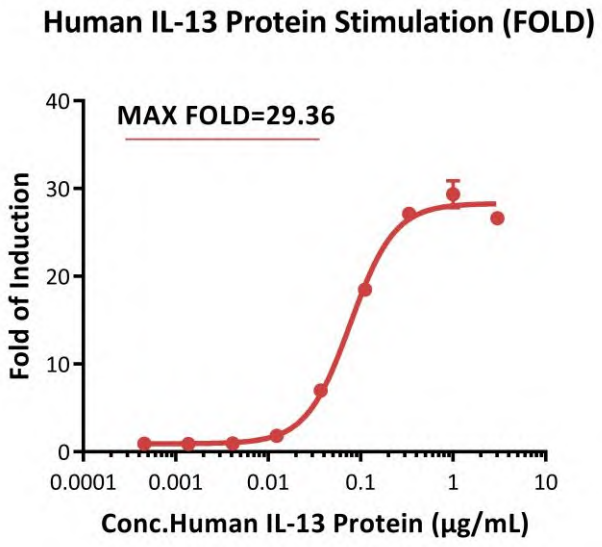

Fig4. Response to human IL-13 protein (FOLD). The Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-13 protein (Cat. No. IL3-H52H4). The max induction fold was approximately 29.36.

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• *Application*

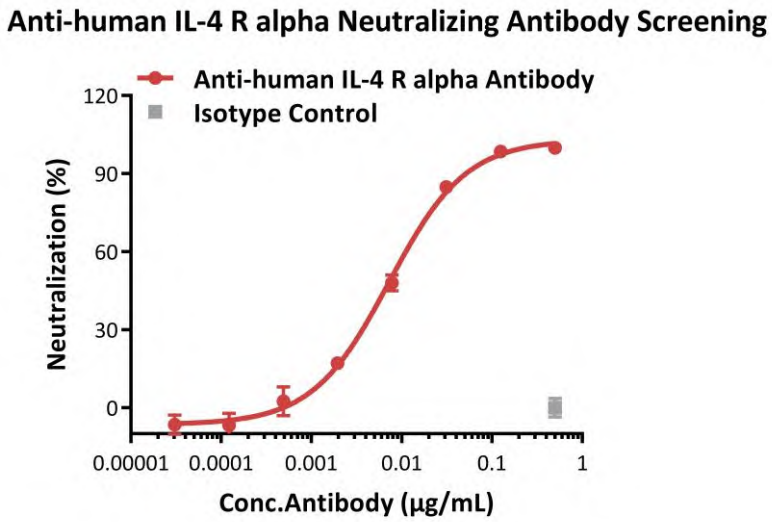

Fig5. Inhibition of human IL-4 protein-induced reporter activity by anti-human IL-4 R alpha neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-4 protein (Cat. No. IL4-H4218) with a final concentration of 0.002 μg/mL. The EC50 of anti-human IL-4 R alpha neutralizing antibody is approximately 0.0073 μg/mL.

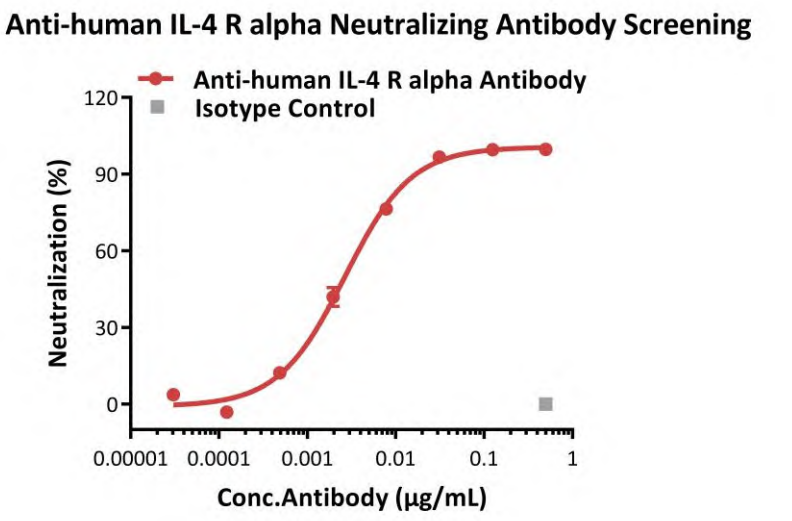

Fig6. Inhibition of human IL-13 protein-induced reporter activity by anti-human IL-4 R alpha neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-13 protein (Cat. No. IL3-H52H4) with a final concentration of 0.1 μg/mL. The EC50 of anti-human IL-4 R alpha neutralizing antibody is approximately 0.0027 μg/mL.

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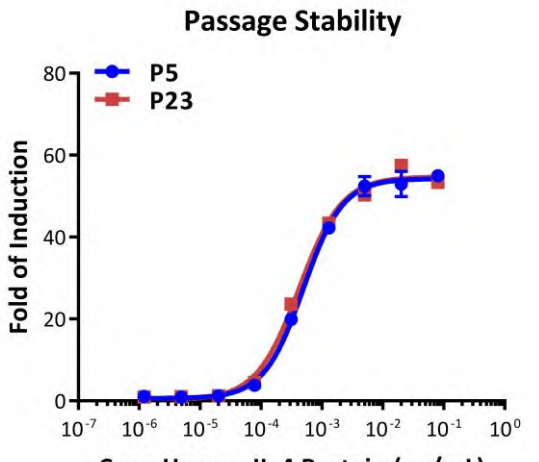
• *Passage Stability*



	P5	P23
EC50 (μg /mL)	0.00050	0.00042
Max Fold	54.99	57.50

Fig7. Passage stability analysis by Signaling Bioassay. The continuously growing Human IL-4 R alpha/IL-13 R alpha 1 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-4 protein. Human IL-4 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 5-23.

• *License Disclosure*

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• *Related Products*

Products	Cat. No.
Human IL-4 Protein, premium grade	IL4-H4218
Human IL-13 Protein, His Tag	IL3-H52H4