Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell Data Sheet

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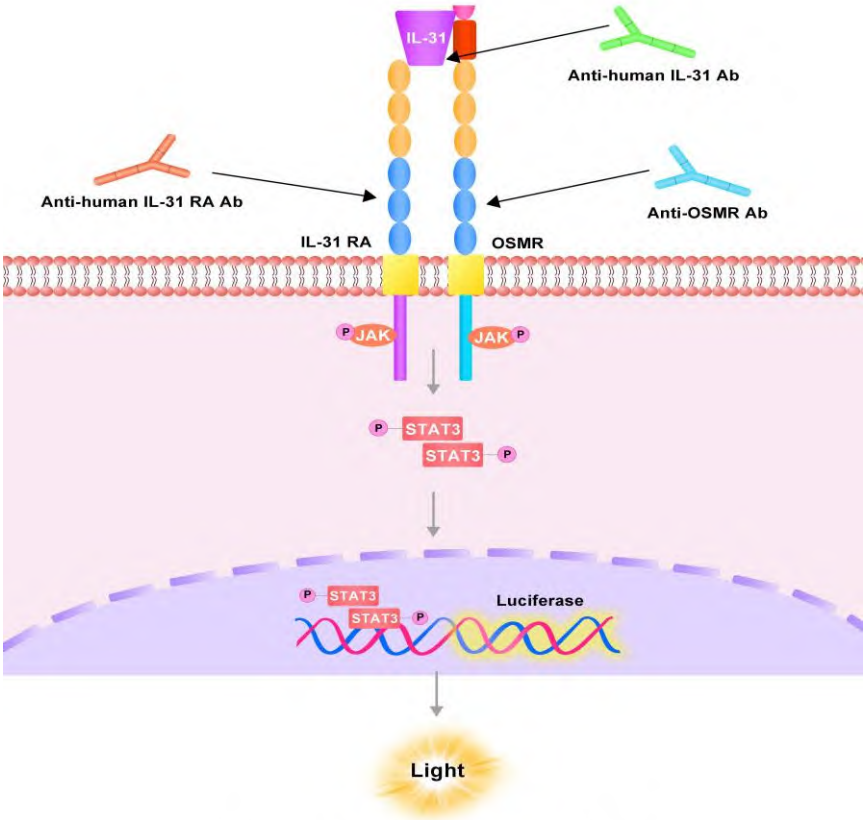
Catalog No.	Size
CHEK-ATF094	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell was engineered to express STAT3 signaling response element, but also express the receptors full length human IL-31 RA (Gene ID: 133396) and OSMR (Gene ID: 9180). When stimulated with human IL-31 protein, the IL-31/IL-31 RA interaction drives STAT3-mediated luminescence. Neutralization of biological effect of human IL-31 protein by corresponding antibody results in a decrease in luminescence.

• *Application*

- Screen for neutralizing antibodies blocking the stimulation of human IL-31 protein.



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• *Cell Line Profile*

Cell line	Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin (40 μg/mL) + Zeocin (20μg/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)
- Hygromycin B (Invitrogen, Cat. No. 10687010)
- Zeocin (Invitrogen, Cat. No. R25001)
- 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 (40 μg/mL), Zeocin (20μg/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)

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• *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_2 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_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.

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• *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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- ***Receptor Assay***

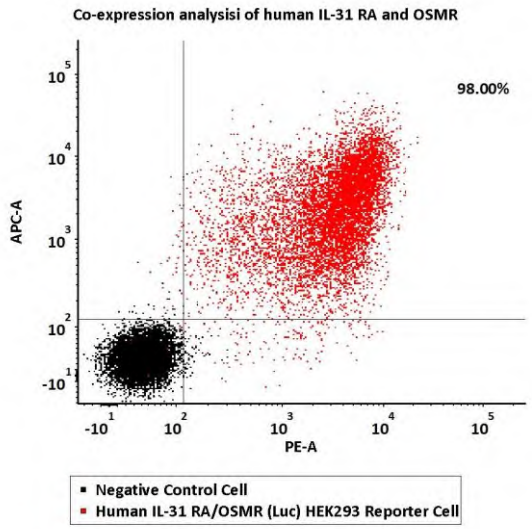

Fig1. Co-expression analysis of human IL-31 RA and OSMR on Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human IL-31 RA antibody and APC-labeled anti-human OSMR antibody.

- ***Signaling Bioassay***

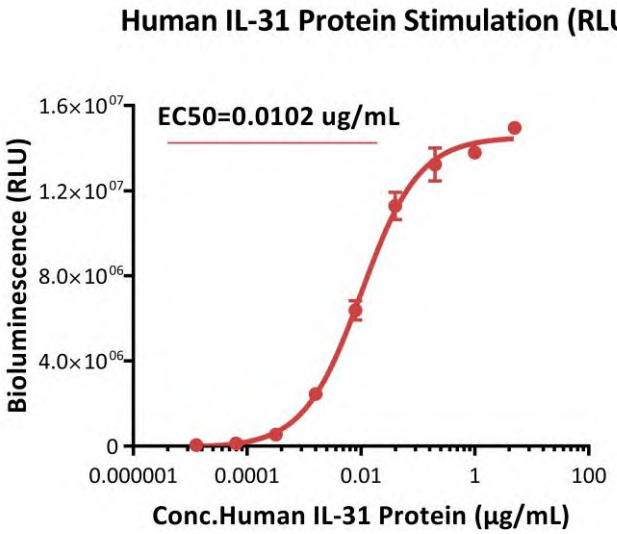

Fig2. Response to human IL-31 protein (RLU). The Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-31 protein (Cat. No. IL1-H5247). The EC50 was approximately 0.0102 μg/mL.

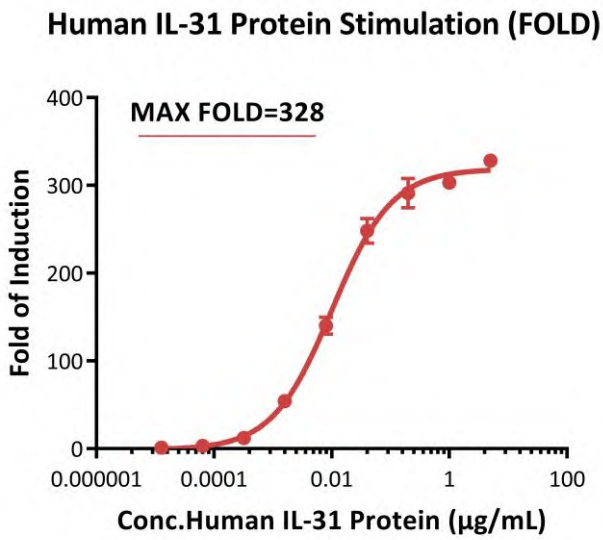

Fig3. Response to human IL-31 protein (FOLD). The Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-31 protein (Cat. No. IL1-H5247). The max induction fold was approximately 328.

- ***Application***

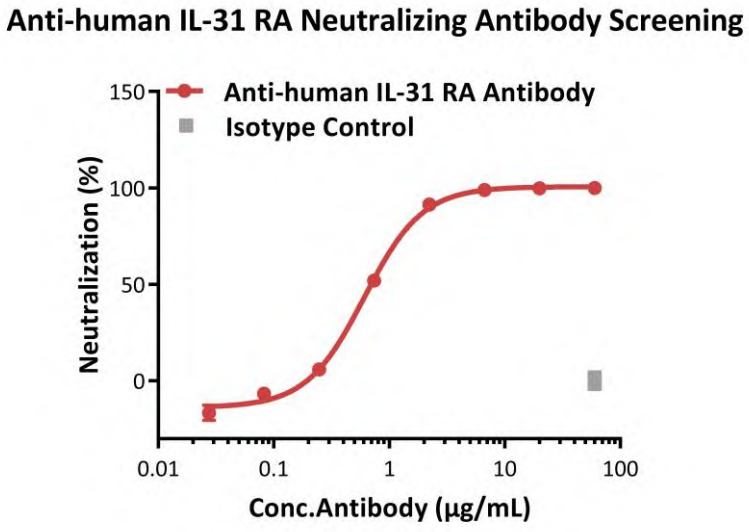

Fig4. Inhibition of human IL-31 protein-induced reporter activity by anti-human IL-31 RA neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-31 protein (Cat. No. IL1-H5247) with a final concentration of 0.001 μg/mL. The EC50 of anti-human IL-31 RA neutralizing antibody is approximately 0.609 μg/mL.

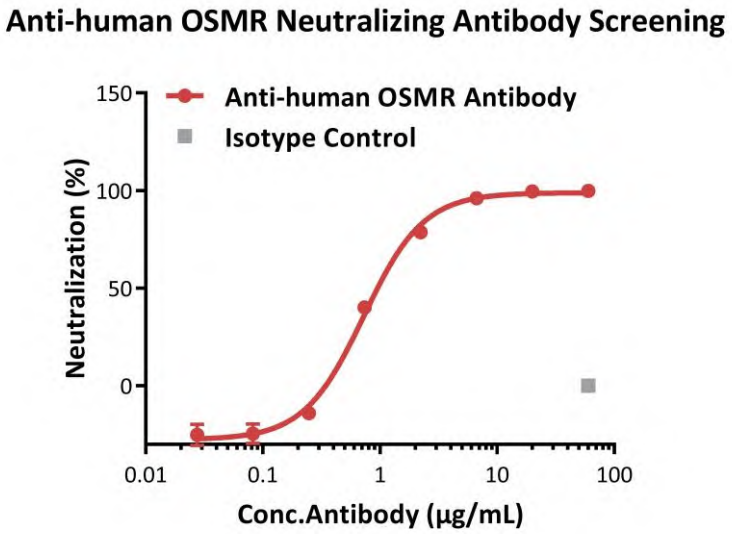

Fig5. Inhibition of human IL-31 protein-induced reporter activity by anti-human OSMR neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-31 protein (Cat. No. IL1-H5247) with a final concentration of 0.001 μg/mL. The EC50 of anti-human OSMR neutralizing antibody is approximately 0.727 μg/mL.

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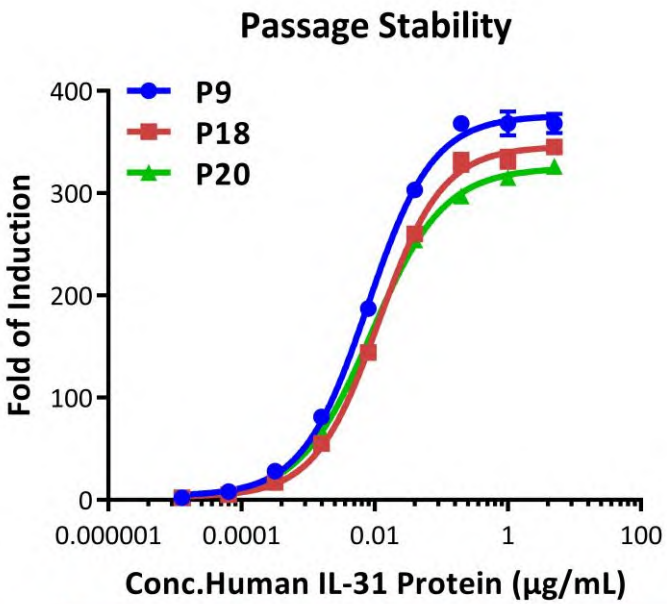
• *Passage Stability*



	P9	P18	P20
EC50 (μg/mL)	0.0079	0.012	0.0090
Max Fold	368	345	326

Fig6. Passage stability analysis by Signaling Bioassay. The continuously growing Human IL-31 RA/OSMR (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-31 protein. Human IL-31 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 9-20.

• *License Disclosure*

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• *Related Products*

Products	Cat. No.
Human IL-31 Protein, His Tag (MALS verified)	IL1-H5247