

## STAT3 (Luc) HEK293 Reporter Cell Data Sheet

### STAT3 (Luc) HEK293 Reporter Cell

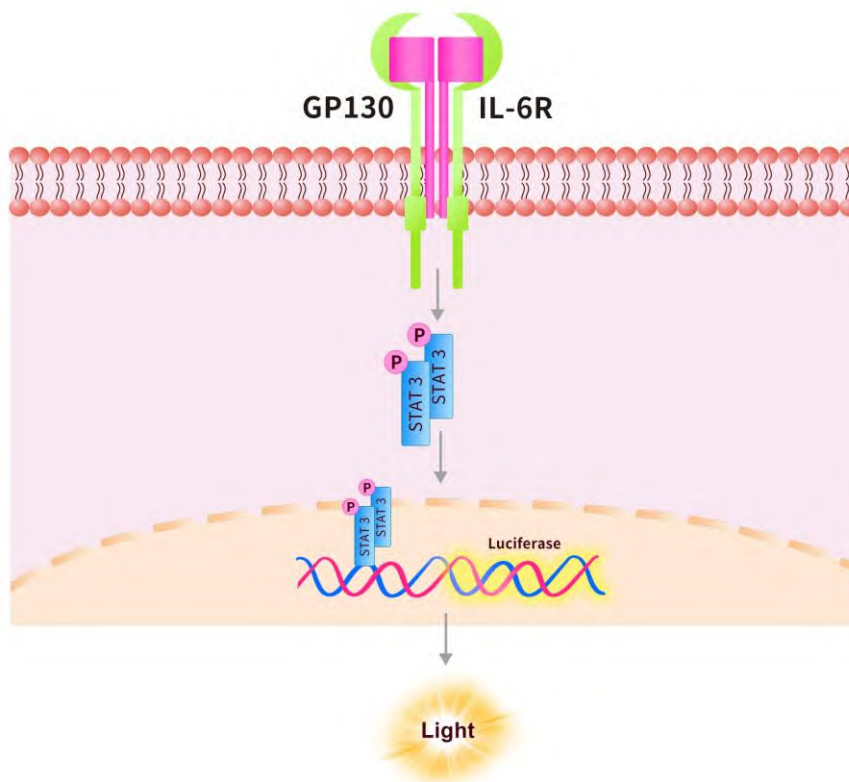
Catalog No.	Size
CHEK-ATF047	2 × (1 vial contains ~5×10 <sup>6</sup> cells)

#### • *Description*

The STAT3 (Luc) HEK293 Reporter Cell was engineered with the STAT3 response element driving luciferase expressing systems. The receptors expressing endogenously or transfected on this reporter cell were activated by corresponding ligands binding, transducing intracellular signals resulting in STAT3-RE mediated luminescence. •

#### • *Application*

- The discovery of activators or inhibitors by the STAT3 signaling bioactivity.
- Transfection host for some receptors concerning the STAT3 signaling pathway.



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## • Cell Line Profile

<b>Cell line</b>	STAT3 (Luc) HEK293 Reporter Cell
<b>Host Cell</b>	HEK293
<b>Property</b>	Adherent
<b>Complete Growth Medium</b>	DMEM + 10% FBS
<b>Selection Marker</b>	Hygromycin (40 µg/mL)
<b>Incubation</b>	37°C with 5% CO <sub>2</sub>
<b>Doubling Time</b>	22-24 hours
<b>Transduction Technique</b>	Lentivirus

## • Materials Required for Cell Culture

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Hygromycin B (Invitrogen, Cat.No.10687010)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Hygromycin (40 µg/mL)
- 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<sub>2</sub> 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<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.

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### • *Cryopreservation*

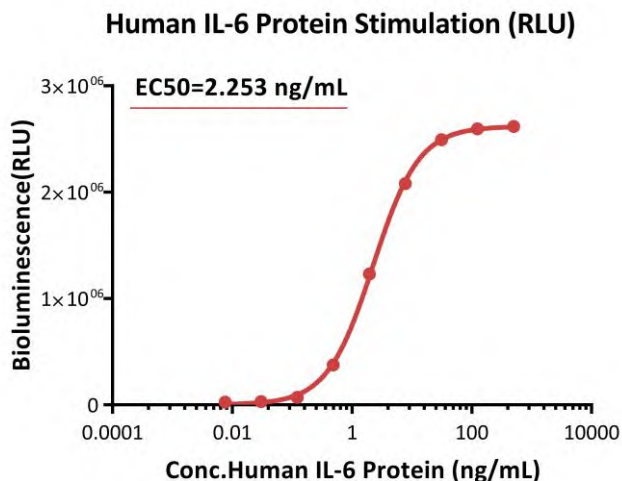
1. Remove and discard spent medium.
2. Detach cells from the cell culture flasks with 0.25% trypsin.
3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
4. Resuspend the cell pellets with complete growth medium and count viable cells.
5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of  $5 \times 10^6$  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  $-80^\circ\text{C}$  freezer overnight, then transferring to liquid nitrogen storage.

### • *Storage*

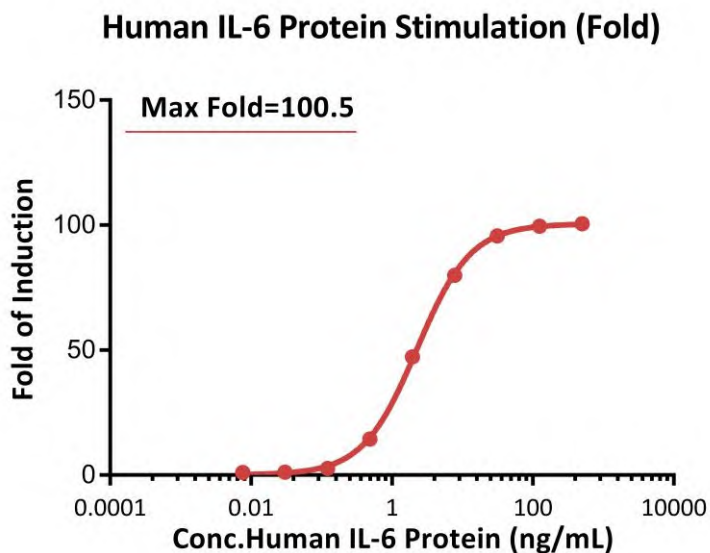
- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

# STAT3 (Luc) HEK293 Reporter Cell Data Sheet

• *Signaling Bioassay*



**Fig1. Response to human IL-6 protein (RLU).** The STAT3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-6 protein (AcroBioSystems, Cat.No.IL6-H4218). The EC50 was approximately 2.2 ng/mL.

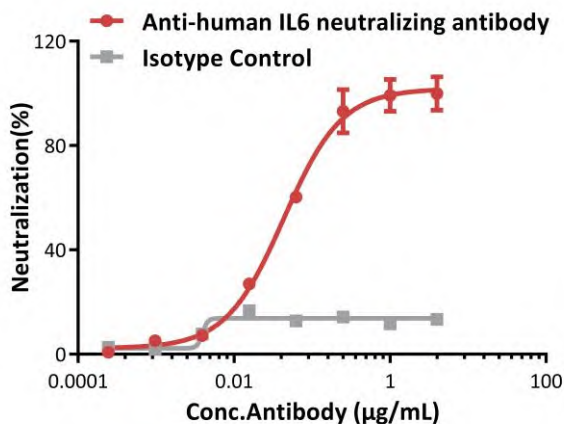


**Fig2. Response to human IL-6 protein (Fold).** The STAT3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-6 protein (AcroBioSystems, Cat.No.IL6-H4218). The max induction fold was approximately 100.5.

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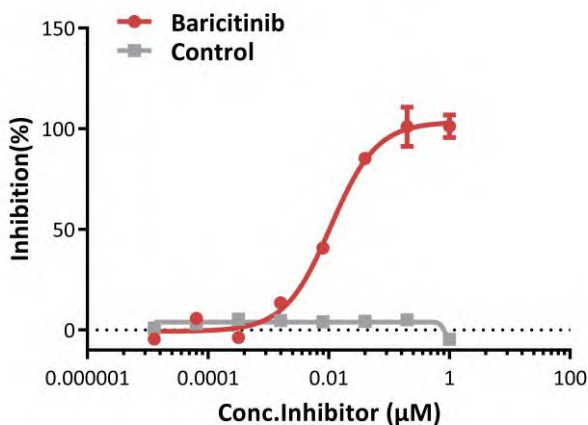
• *Application*

## Anti-human IL-6 Neutralizing Antibody Screening



**Fig3. Inhibition of human IL-6 protein-induced reporter activity by anti-human IL-6 neutralizing antibody.** This reporter cell was incubated with serial dilutions of antibodies in the presence of human IL-6 protein (AcroBiosystems, Cat.No.IL6-H4218) with a final concentration of 2.5 ng/mL. The EC50 of anti-human IL-6 neutralizing antibody is approximately 0.04 µg/mL.

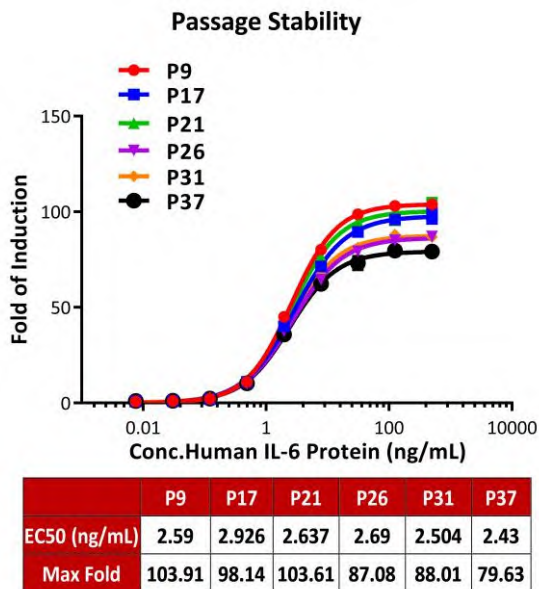
## JAK Inhibitor Screening



**Fig4. Inhibition of human IL-6 protein-induced reporter activity by JAK inhibitor.** This reporter cell was incubated with serial dilutions of JAK inhibitors in the presence of human IL-6 protein with a final concentration of 2.5 ng/mL. The EC50 of JAK inhibitor (Baricitinib) was approximately 0.0108 µM.

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• *Passage Stability*



**Fig5. Passage stability analysis by Signaling Bioassay.** The continuously growing STAT3 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-6 protein. Human IL-6 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 9-37.

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## • *License Disclosure*

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## • *Related Products*

### Products

Human IL-6 Protein, premium grade

### Cat.No.

IL6-H4218