

Limited Use & License Disclosure

BY USE OF THIS PRODUCT, RESEARCHER AGREES TO BE BOUND BY THE FOLLOWING TERMS OF LIMITED USE OF THIS CELL LINE PRODUCT.

- If the researcher is not willing to accept the terms of limited use of this cell line product, and the product is unused, ACRO will accept return of the unused product.
- Researchers may use this product for research use only, no commercial use is allowed.

 "Commercial use" means any and all uses of this product and derivatives by a party for profit or other consideration and may include but is not limited to use in: (1) product manufacture; and (2) to provide a service, information or data; and/or resale of the product or its derivatives, whether or not such product or derivatives are resold for use in research.
- This cell line is neither intended for any animal or human therapeutic purposes nor for any direct human in vivo use. You have no right to share, modify, transfer, distribute, sell, sublicense, or otherwise make the cell line available for use to other researchers, laboratories, research institutions, hospitals, universities, or service organizations.
- ACROBIOSYSTEMS MAKES NO WARRANTIES OR REPRESENTATIONS OF ANY KIND,
 EITHER EXPRESSED OR IMPLIED, WITH RESPECT TO THE SUITABILITY OF THE CELL
 LINE FOR ANY PARTICULAR USE.
- ACROBIOSYSTEMS ACCEPTS NO LIABILITY IN CONNECTION WITH THE HANDLING OR USE OF THE CELL LINE.
- Modifications of the cell line, transfer to a third party, or commercial use of the cell line may require a separate license and additional fees. Please contact order.cn@acrobiosystems.com for further details.



Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell

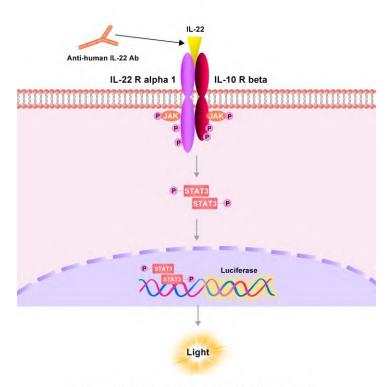
| Catalog No. | Size |
|-------------|--|
| CHEK-ATF167 | $2 \times (1 \text{ vial contains } \sim 5 \times 10^6 \text{ cells})$ |

• Description

The Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell was engineered to not only express STAT3 signaling response element, but also express the receptors full length human IL-22 R alpha 1 (Uniprot: Q8N6P7) and IL-10 R beta (Uniprot: Q08334). When stimulated with human IL-22 protein, receptor-mediated signaling can drive STAT3-mediated luminescence. Neutralization of biological effect of human IL-22 protein by corresponding antibody results in a decrease in luminescence.

• Application

- Screen for neutralizing antibodies blocking the stimulation of human IL-22 protein
- Bioactivity detection of human IL-22 fusion protein



Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell



• Cell Line Profile

| Cell line | Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell |
|------------------------|---|
| Host Cell | HEK293 |
| Property | Adherent |
| Complete Growth Medium | DMEM + 10% FBS |
| Selection Marker | Puromycin (2 μg/mL) + Hygromycin B (40 μg/mL) + Zeocin (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)
- Zeocin (Invitrogen, Cat. No. R25001)

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 (40 μg/mL), Zeocin (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)



• 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.



• 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.



• Receptor Assay

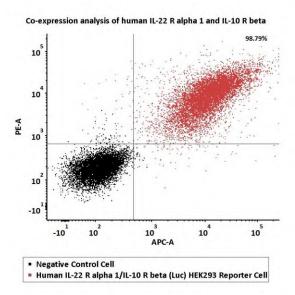


Fig1. Co-expression analysis of human IL-22 R alpha 1 and IL-10 R beta on Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell or negative control cell using anti-human IL-22 antibody followed by staining with PE anti-human IgG Fc antibody and APC-labeled anti-IL-10 R beta antibody.

• Signaling Bioassay

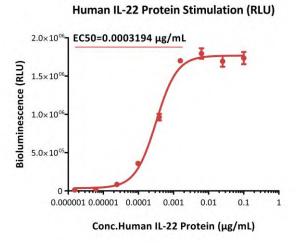


Fig2. Response to human IL-22 protein (RLU). The Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-22 protein (Cat. No. IL2-H524a). The EC50 was approximately $0.0003194 \, \mu g/mL$.



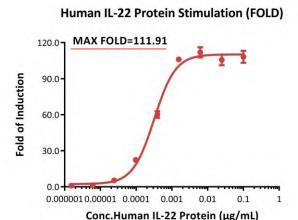


Fig3. Response to human IL-22 protein (FOLD). The Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human IL-22 protein (Cat. No. IL2-H524a). The max induction fold was approximately 111.91.

• Application

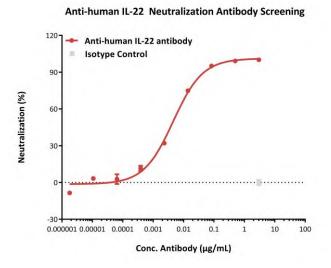
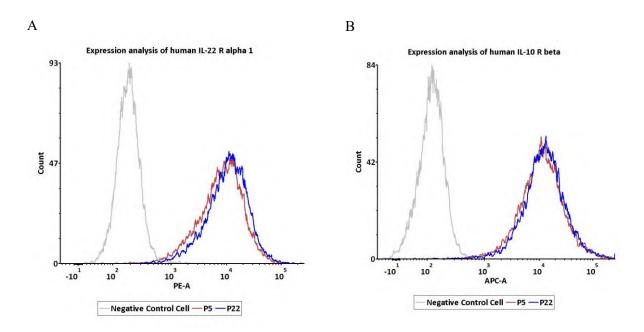


Fig4. Inhibition of human IL-22 protein-induced reporter activity by anti-human IL-22 neutralizing antibody. The Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell was incubated with serial dilutions of antibodies in the presence of human IL-22 protein (Cat. No. IL2-H524a) with a final concentration of $0.0005 \, \mu \text{g/mL}$. The EC50 of anti-human IL-22 neutralizing antibody is approximately $0.004567 \, \mu \text{g/mL}$.



• Passage Stability



| Passage | MFI for IL-22 R alpha 1 (PE) | MFI for IL-10 R beta (APC) |
|---------|------------------------------|----------------------------|
| P5 | 8368.39 | 10859.46 |
| P22 | 10555.09 | 12303.22 |

Fig5. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human IL-22 R alpha 1 and IL-10 R beta on Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across passage 5-22. (A) Human IL-22 R alpha 1 expression analysis. (B) Human IL-10 R beta expression analysis.



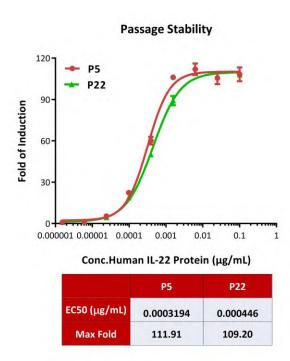


Fig6. Passage stability analysis by Signaling Bioassay. The continuously growing Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of IL-22 protein. IL-22 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 5-22.



• 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 |
| HEK293/Human CD40 Ligand / TNFSF5 Stable Cell Line | CHEK-ATP041 |
| HEK293/Human OX40 / TNFRSF4 / CD134 Stable Cell Line | CHEK-ATP053 |
| HEK293/Human OX40 Ligand / TNFSF4 Stable Cell Line | CHEK-ATP054 |
| Human IL-5 R alpha/CD131 (Luc) HEK293 Reporter Cell | CHEK-ATF074 |
| HEK293/FcRn (FCGRT & B2M) Cell Line | CHEK-ATP079 |
| Human IL-21 R (Luc) HEK293 Reporter Cell | CHEK-ATF051 |
| 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 |
| CHO/Human TSHR Stable Cell Line | SCCHO-ATP085 |
| HEK293/Human TSHR Stable Cell Line | CHEK-ATP086 |
| 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 CD40 (Luc) HEK293 Reporter Cell | CHEK-ATF097 |
| Human IL-7 R alpha/CD132 (Luc) HEK293 Reporter Cell | CHEK-ATF099 |
| NIH-3T3/Human IGF-1 R Stable Cell Line Development Service | CNIH-ATP102 |
| Human HVEM (Luc) HEK293 Reporter Cell | CHEK-ATF105 |
| Human BTLA (Luc) Jurkat Reporter Cell | SCJUR-STF106 |
| Human IGF-1 R (Luc) HEK293 Reporter Cell | CHEK-ATF107 |
| 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 |
| Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell | CHEK-ATF133 |
| Human OX40 (Luc) HEK293 Reporter Cell | CHEK-ATF135 |
| Human IL-2 R beta/IL-2 R gamma (Luc) HEK293 Reporter Cell | CHEK-ATF136 |
| HEK293/Human TL1A Stable Cell Line | CHEK-ATP142 |
| Human IL-23 R/IL-12 R beta 1(Luc) HEK293 Reporter Cell | CHEK-ATF166 |



• Related Products

| <u>Products</u> | Cat.No. |
|---|--------------|
| Human TSHR (Luc) HEK293 Reporter Cell | CHEK-ATF187 |
| CHO/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line | SCCHO-ATP193 |
| CHO/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line | CHEK-ATF194 |
| MDCK/Mouse FCGRT-P2A-mGFP&B2M Stable Cell Line Development | SCMDC-ATP196 |
| Service | |
| Human TACI (Luc) HEK293 Reporter Cell | CHEK-ATF197 |
| HEK293/Membrane-Bound Human TL1A Stable Cell Line | CHEK-ATF198 |
| Human IL-2 R alpha & IL-2 R beta & IL-2 R gamma (Luc) HEK293 Reporter | CHEK-ATF201 |
| Cell | |
| Human IL-1 R1 & IL-1 RAcP (Luc) HEK293 Reporter Cell | CHEK-ATF202 |
| Raji/Membrane-Bound Human TL1A Stable Cell Line | SCRAJ-STT204 |
| HEK293/Human MRGPRX2 Stable Cell Line | CHEK-ATP214 |
| CHO/Human MRGPRX2 Stable Cell Line | SCCHO-ATP215 |
| Human TPO R (Luc) HEK293 Reporter Cell | CHEK-ATF226 |