Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

➢ *LIMITED USE AND LICENSE STATEMENT*

HEK293 Cell Line Products

By using any HEK293 cell line product purchased from ACRO Biosystems or its affiliates (collectively, "ACROBiosystems"), including the original 293 cells and any modified derivatives thereof (collectively, the "Product"), you (the "Customer") agree to be bound by the following terms of limited use. If you are not willing to accept these terms and the Product remains unused, please contact our customer service at customerservice@acrobiosystems.com to arrange for return of the unused Product for a refund.

1. Scope of Use

- For the use of the Product **worldwide** (excluding Mainland China, Hong Kong, Macao and Taiwan regions), **Customer may use the Product for (i) internal research; (ii) drug discovery and assay development; (iii) internal quality assurance testing for biologic drugs and vaccines; and (iv) lot releasing assay for biologic drugs and vaccines, whether conducted by a commercial or non-commercial entity.** Unless a separate sublicense has been obtained, it is **NOT** permitted to use the Product in (i) product manufacturing; (ii) provision of service, information, or data to an unaffiliated third party for a fee; (iii) resale of the Product or its derivatives, whether or not such Product or derivatives are resold for use in research or (iv) other commercial use not expressly permitted above.
- For the use of the Product in **Greater China region** (including Mainland China, Hong Kong, Macao and Taiwan regions), **Customer may use the Product for internal research use only**. Unless a separate sublicense has been obtained, it is **NOT** permitted to use the Product for any Commercial Use. For the purpose of this paragraph, "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: (i) product manufacture; (ii) provision of service, information or data to a third party for a fee; (iii) resale of the product or its derivatives, whether or not such Product or derivatives are resold for use in research; (iv) quality assurance testing for biologic drugs and vaccines; (v) quality control assays; (vi) lot releasing assay of biologic drugs and vaccines.
- If you require a sublicense for any use beyond the limited use permitted herein, including but not limited to commercial use, CRO/service provider activities for a fee, or any product manufacturing activities, please contact the local sales team of ACROBiosystems for further details.
- The Product is not intended for any animal or human therapeutic purposes, nor for any direct human in vivo use. The Product shall not be used in or administered to humans.

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

➢ *LIMITED USE AND LICENSE STATEMENT*

2. Transfer, Distribution and Sublicensing Restrictions

Unless expressly permitted under a separate written agreement with/via ACROBiosystems, you have **NO** right to (i) share, transfer, distribute, sell, resell, sublicense, or otherwise make the Product or its derivatives available for use to any third party, including but not limited to other researchers, laboratories, departments, research institutions, hospitals, universities, contract research organizations (CROs), contract development and manufacturing organizations (CDMOs), or any other commercial entities; or (ii) use the Product or its derivatives to perform fee-for-service activities or to provide services, information, or data to any third party for monetary or other consideration.

3. Modification and Derivatives

Customers have **No** right to modify, engineer, genetically alter, or create derivatives of the Product. Any such modification or derivative requires a separate license and additional fees. Please contact our local sales team for further details.

4. No Warranty

ACROBiosystems makes no warranties or representations of any kind, either expressed or implied, with respect to the merchantability, fitness or suitability of the Product for any particular purpose.

5. Limitation of Liability

To the fullest extent permitted by law, in no event shall ACROBiosystems or its licensors be liable for any use by Customer of the Product, any derivative thereof, or any material treated therewith for any losses, costs, claims, damages, or liability of whatsoever kind or nature which may arise from or in connection with the use of the Product.

6. Compliance with Laws

Customer agrees to use the Product in compliance with all applicable laws, regulations, and governmental guidelines applicable to the Product, any derivative thereof, and any materials treated therewith.

ACKNOWLEDGMENT

By using this Product, Customer acknowledges that he/she has read, understood, and agreed to be bound by the terms and conditions of this Limited Use and License Statement. If Customer does not agree to comply with these terms, Customer shall not open or use the Product and shall contact ACROBiosystems to arrange for return of the unused Product.

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

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

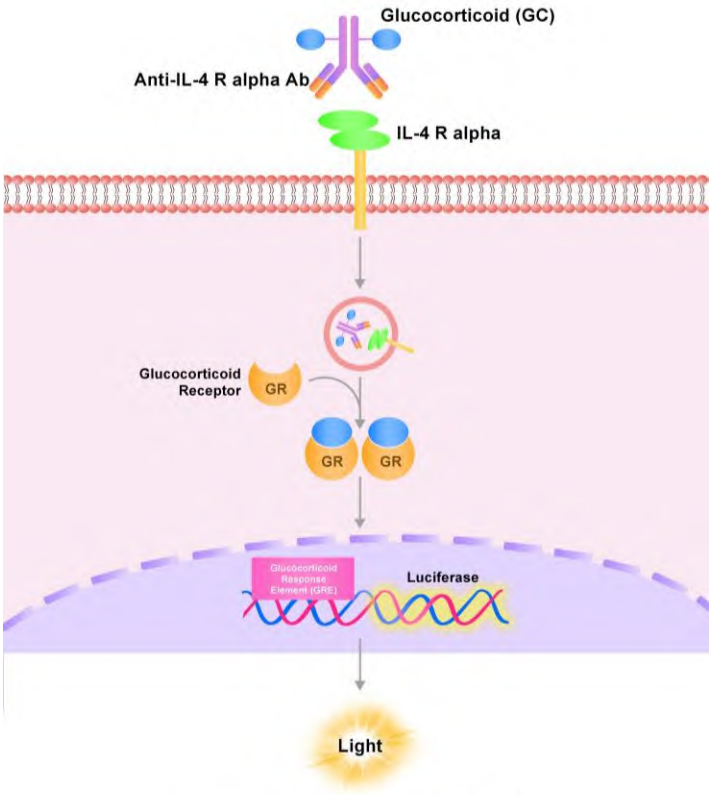
Catalog No.	Size
CHEK-ATF304	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human IL-4 R alpha (Luc) HEK293 Reporter Cell expressing human glucocorticoid receptor (GR) was engineered to not only express the glucocorticoid response element (GRE), but also express the receptor full length human IL-4 R alpha (Uniprot: P24394-1). In the absence of the IL-4 R alpha-ADC (via an anti-IL-4 R alpha antibody conjugated to a glucocorticoid agonist to form an anti-IL-4 R alpha antibody-drug conjugate), the human GR is not activated and luminescence signal is low. In the presence of the IL-4 R alpha-ADC, the glucocorticoid agonist released from the IL-4 R alpha-ADC can bind the human GR, resulting in the GRE-mediated luminescence.

• *Application*

• Screen for the IL-4 R alpha-targeting antibody-drug conjugate (IL-4 R alpha-ADC), formed by conjugating an anti-IL-4 R alpha antibody with a glucocorticoid agonist



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

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

• *Cell Line Profile*

Cell line	Human IL-4 R alpha (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 μg/mL) + Hygromycin B (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)

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 (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)
- CO_2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

• *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℃ with 5% CO_2 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℃ with 5% CO_2 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.

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

• *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.

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

- ***Receptor Assay***

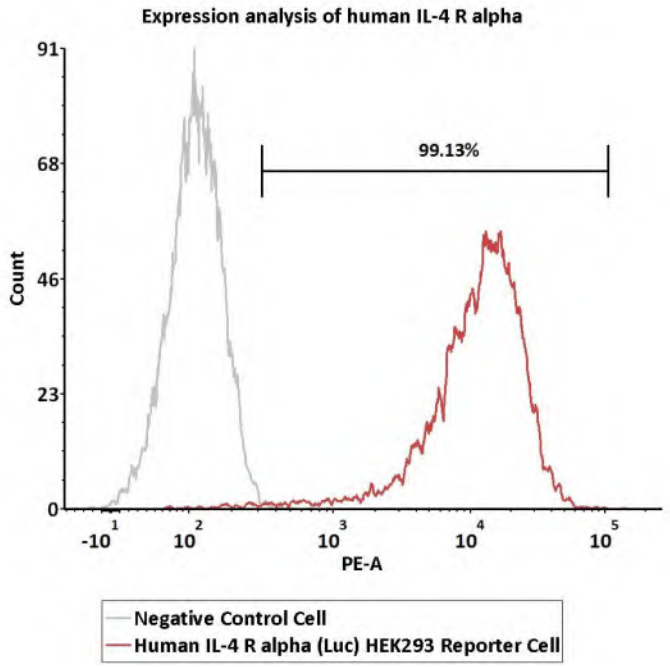


Fig1. Expression analysis of human IL-4 R alpha on Human IL-4 R alpha (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human IL-4 R alpha (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human IL-4 R antibody.

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

• ***Signaling Bioassay***

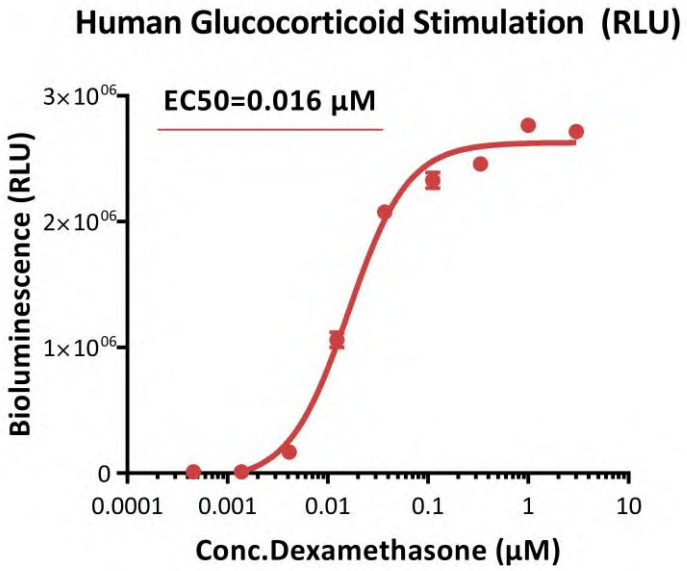


Fig2. Response to human glucocorticoid receptor agonist (RLU). This reporter cell was incubated with serial dilutions of human glucocorticoid receptor agonist. The EC50 of human glucocorticoid receptor agonist (Dexamethasone) was approximately 0.016 μM.

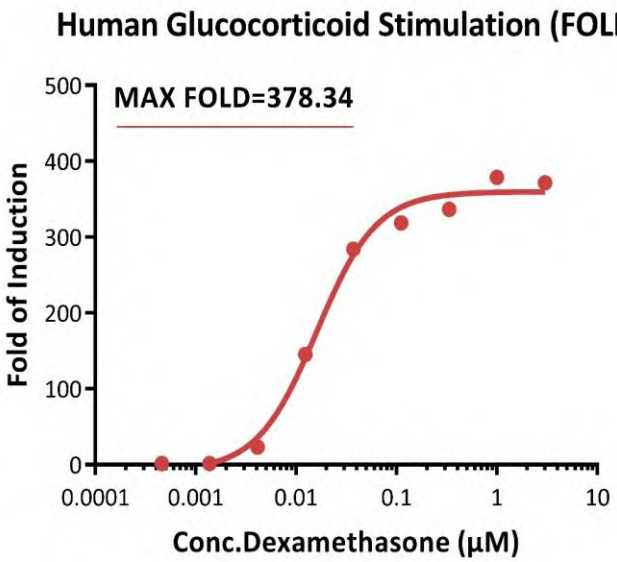


Fig3. Response to human glucocorticoid receptor agonist (FOLD). This reporter cell was incubated with serial dilutions of human glucocorticoid receptor agonist. The max induction fold of human glucocorticoid receptor agonist (Dexamethasone) was approximately 378.34.

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

• *Related Products*

Products	Cat.No.
Human Glucocorticoid Receptor (Luc) HEK293 Reporter Cell	CHEK-ATF264
HEK293/hClaudin-18.2 Cell Line	CHEK-ATP033
HEK293/hGPRC5D Cell Line	CHEK-STP042
HEK293/Human TROP-2 Stable Cell Line	CHEK-ATP036
HEK293/Human Nectin-4 Stable Cell Line	CHEK-ATP035
HEK293/Human Anti-CD19 Stable Cell Line	CHEK-ATS056
CHO/Human GPRC5D Stable Cell Line	CCHO-STP078
HEK293/Human CEACAM5 Stable Cell Line	CHEK-ATP083
HEK293/Human ROR1 Stable Cell Line	CHEK-ATP084
HEK293/Human Transferrin R Stable Cell Line	CHEK-ATP089
HEK293/Human DLL3 Stable Cell Line	CHEK-ATP090
HEK293/Human FOLR1 Stable Cell Line	CHEK-ATP091
HEK293/Human Glypican-3 (GPC3) Stable Cell Line	CHEK-ATP092
CHO/Human DLL3 Stable Cell Line	SCCHO-ATP111
CHO/Human Glypican-3 (GPC3) Stable Cell Line	SCCHO-ATP112
HEK293/Human Transferrin Stable Cell Line	CHEK-ATP115
HEK293/Human NAPI-IIb Stable Cell Line	CHEK-ATP116
HEK293/Human Mesothelin Stable Cell Line	CHEK-ATP119
CHO/Human Mesothelin Stable Cell Line	SCCHO-ATP120
CHO/Human STEAP1 Stable Cell Line	SCCHO-ATP121
HEK293/Human ENPP3 Stable Cell Line	CHEK-ATP122
HEK293/Human LRRC15 Stable Cell Line	CHEK-ATP123
HEK293/Human Claudin-1 Stable Cell Line	CHEK-ATP124
HEK293/Human Integrin alpha V beta 6 Stable Cell Line	CHEK-ATP125
HEK293/Human B7-H4 Stable Cell Line	CHEK-ATP126
HEK293/Human Cadherin-6 Stable Cell Line	CHEK-ATP127
HEK293/Human LY6G6D Stable Cell Line	CHEK-ATP137
HEK293/Human Claudin-6 Stable Cell Line	CHEK-ATP138

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

• *Related Products*

Products	Cat.No.
HEK293/Human Claudin-9 Stable Cell Line	CHEK-ATP139
CHO/Human c-MET Stable Cell Line	SCCHO-ATP141
HEK293/Human c-MET Stable Cell Line	CHEK-ATP146
HEK293/Human EGF R Stable Cell Line	CHEK-ATP148
HEK293/Human ErbB3 Stable Cell Line	CHEK-ATP149
HEK293/Human ErbB2 Stable Cell Line	CHEK-ATP150
HEK293/Human uPAR Stable Cell Line	CHEK-ATP151
CHO/Human uPAR Stable Cell Line	SCCHO-ATP152
HEK293/Human CD19 Stable Cell Line	CHEK-ATP003
HEK293/Human STEAP1 Stable Cell Line	CHEK-ATP154
CHO/Human B7-H3 (4Ig) Stable Cell Line	SCCHO-ATP169
CHO/Human CD79A&CD79B Stable Cell Line	SCCHO-ATP170
CHO/Human CD79B Stable Cell Line	SCCHO-ATP171
HEK293/Human Cadherin-17 Stable Cell Line	CHEK-ATP173
HEK293/Human EpCAM Stable Cell Line	CHEK-ATP175
HEK293/Human TPBG Stable Cell Line	CHEK-ATP176
CHO/Cynomolgus Glypican-3 (GPC3) Stable Cell Line	SCCHO-ATP179
HEK293/Human GUCY2C Stable Cell Line	CHEK-ATP182
HEK293/Human SEZ6 Stable Cell Line	CHEK-ATP183
HEK293/Human FAP Stable Cell Line	CHEK-ATP184
HEK293/Human PSMA Stable Cell Line	CHEK-ATP185
HEK293/Human PTK7 Stable Cell Line	CHEK-ATP186
HEK293/Human MCAM Stable Cell Line	CHEK-ATP195
HEK293/Human GPC3 Δ HS Stable Cell Line	CHEK-ATP212
HEK293/Human c-MET&ErbB3 Stable Cell Line	CHEK-ATP217
HEK293/Human BCMA Stable Cell Line	CHEK-ATP218
Raji/Human CD20 Knockout Stable Cell Line	SCRAJ-STT227
CHO/Human CDCP1 (R368A, K369A) Stable Cell Line	CCHO-ATP234

Human IL-4 R alpha (Luc) HEK293 Reporter Cell Data Sheet

• *Related Products*

Products	Cat.No.
CHO/Human CDCP1 (NTF&CTF) Stable Cell Line	CCHO-ATP235
Raji/Human CD19 & CD20 Double Knockout Stable Cell Line	CRAJ-STK238
HEK293/Human Tissue Factor Stable Cell Line	CHEK-ATP240
CHO/Human PSMA Stable Cell Line	CCHO-ATP246
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+)	CJUR-STF247
CHO/Human Integrin alpha V beta 6 Stable Cell Line	CCHO-ATP254
HEK293/Human CD133 Stable Cell Line	CHEK-ATP255
Jurkat/Luc Stable Cell Line	CJUR-STP258
HEK293/Human FGF R2 (IIIb) Stable Cell Line	CHEK-ATP259
HEK293/Human STEAP2 Stable Cell Line	CHEK-ATP263
CHO/Human PD-1 Stable Cell Line	CCHO-ATP266
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+ CD8+)	CJUR-STK270