

HEK293/Human LGR5 Stable Cell Line 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.

HEK293/Human LGR5 Stable Cell Line 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.

HEK293/Human LGR5 Stable Cell Line Data Sheet

HEK293/Human LGR5 Stable Cell Line

Catalog No.	Size
CHEK-ATP327	1 vial contains $\sim 5 \times 10^6$ cells

• *Description*

The HEK293/Human LGR5 Stable Cell Line was engineered to express the receptor full length human LGR5 (Uniprot: O75473-1). Surface expression of human LGR5 was confirmed by flow cytometry.

• *Application*

- Useful for cell-based LGR5 binding assay

• *Cell Line Profile*

Cell line	HEK293/Human LGR5 Stable Cell Line
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Hygromycin B (20 μ g/mL)
Incubation	37°C with 5% CO ₂
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

HEK293/Human LGR5 Stable Cell Line Data Sheet

• *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), 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)
- 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, 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)
- CO₂ Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

HEK293/Human LGR5 Stable Cell Line 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°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 70-80% 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 70-80% 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.

HEK293/Human LGR5 Stable Cell Line Data Sheet

• *Cryopreservation*

1. When the cell culture reaches 70-80% 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.

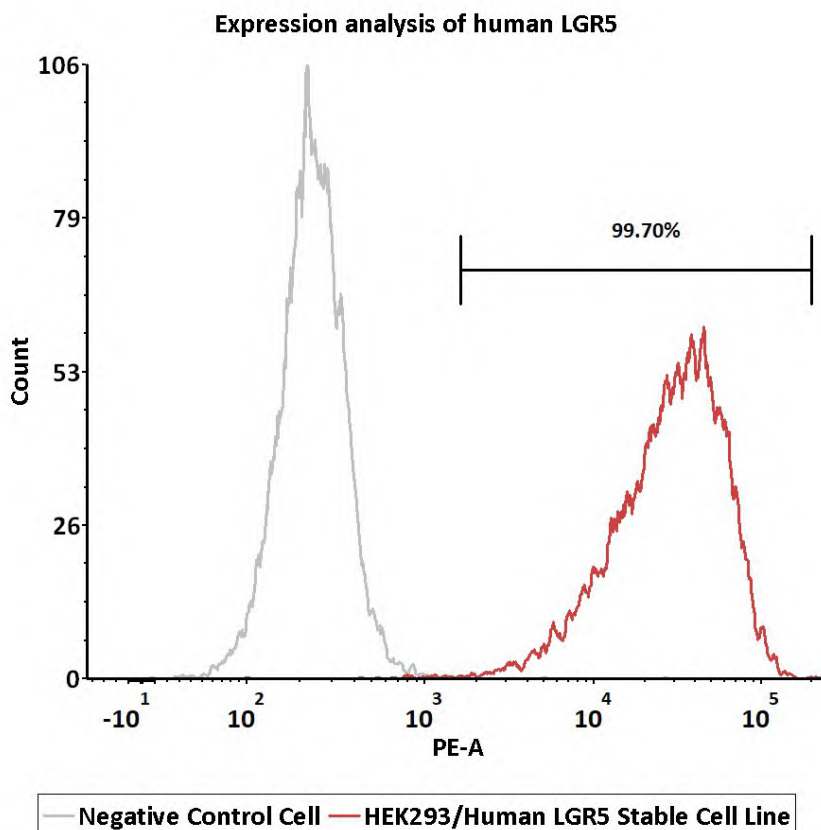
Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *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.

HEK293/Human LGR5 Stable Cell Line Data Sheet

• Receptor Assay



Catalog No.	Stable Cell Line	MFI for LGR5 (PE)
NA	Negative Control Cell	225.83
CHEK-ATP327	HEK293/Human LGR5 Stable Cell Line	29651.67

Fig1. Expression analysis of human LGR5 on HEK293/Human LGR5 Stable Cell Line by FACS. Cell surface staining was performed on HEK293/Human LGR5 Stable Cell Line or negative control cell using PE-labeled anti-human LGR5 antibody.

HEK293/Human LGR5 Stable Cell Line Data Sheet

• *Related Products*

Products

Cat. No.

Human EGFR & LGR5 (SRE-Luc) HEK293 Reporter Cell	CHEK-ATF288
Human VEGF R2 (Luc) HEK293 Reporter Cell	CHEK-ATF044
NF-κB (Luc) HEK293 Reporter Cell	CHEK-ATF048
Human EGF R (Luc) HEK293 Reporter Cell	CHEK-ATF049
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
HEK293/Human CCR5 Stable Cell Line	CHEK-ATP043
HEK293/Human SIRP alpha Stable Cell Line	CHEK-ATP051
HEK293/Human CD20 Stable Cell Line	CHEK-ATP034
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human TMPRSS2-HA-P2A-mGFP Stable Cell Line	CHEK-ATP101
NF-κB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
HEK293/Human CCR8 Stable Cell Line	CHEK-ATP140
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
Human BMP (Luc) HEK293 Reporter Cell	CHEK-ATF188
HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP199
HEK293/Human IDH1(132R)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP200