HEK293/Human GLP-1R Stable Cell Line (High Expression) Data Sheet

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Catalog No.	Size
CHEK-ATP160	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The HEK293/Human GLP-1R Stable Cell Line was engineered to express the receptor full length human GLP-1R (Uniprot: P43220), with different levels of GLP-1R expression (High, Medium, Low). Surface expression of human GLP-1R was confirmed by flow cytometry.

• *Application*

- Useful for cell-based GLP-1R binding assay
- Screen for human GLP-1R agonists based on cAMP accumulation assay

• *Cell Line Profile*

Cell line	HEK293/Human GLP-1R Stable Cell Line (High Expression)
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Hygromycin B (20 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

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• *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)
- 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)

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• *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% 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°C 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.

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• *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.

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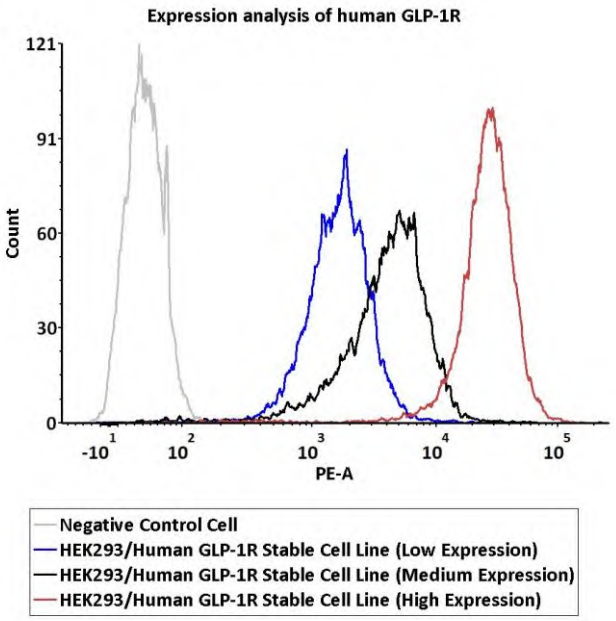
- ***Receptor Assay***



Catalog No.	Stable Cell Line	MFI for GLP-1R (PE)
CHEK-ATP162	HEK293/Human GLP-1R Stable Cell Line (Low Expression)	1604.76
CHEK-ATP161	HEK293/Human GLP-1R Stable Cell Line (Medium Expression)	4208.08
CHEK-ATP160	HEK293/Human GLP-1R Stable Cell Line (High Expression)	26203.40

Fig1. Expression analysis of human GLP-1R on HEK293/Human GLP-1R Stable Cell Line by FACS. Cell surface staining using PE-labeled anti-human GLP-1R antibody was performed on HEK293/Human GLP-1R Stable Cell Line with different expression levels: HEK293/Human GLP-1R Stable Cell Line (Low Expression); HEK293/Human GLP-1R Stable Cell Line (Medium Expression); HEK293/Human GLP-1R Stable Cell Line (High Expression).

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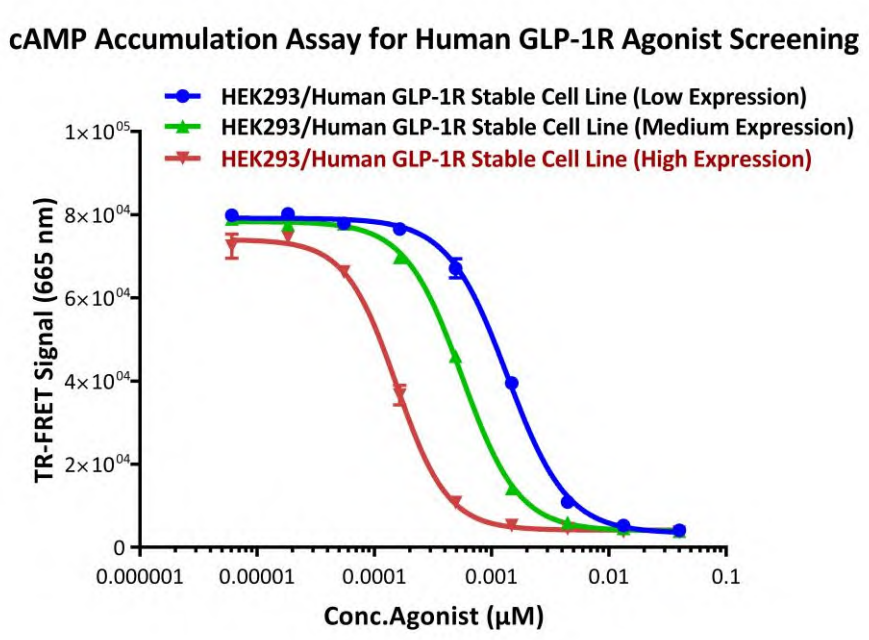
• *Application*



Catalog No.	Stable Cell Line	EC50 (μM)
CHEK-ATP162	HEK293/Human GLP-1R Stable Cell Line (Low Expression)	0.00136
CHEK-ATP161	HEK293/Human GLP-1R Stable Cell Line (Medium Expression)	0.0005579
CHEK-ATP160	HEK293/Human GLP-1R Stable Cell Line (High Expression)	0.0001544

Fig2. cAMP accumulation assay for human GLP-1R agonist screening. HEK293/Human GLP-1R Stable Cell Line (Low Expression), HEK293/Human GLP-1R Stable Cell Line (Medium Expression) and HEK293/Human GLP-1R Stable Cell Line (High Expression) were stimulated with Tirzepatide, respectively. The EC50 of Tirzepatide on HEK293/Human GLP-1R Stable Cell Line (High Expression) was approximately 0.0001544 μM.

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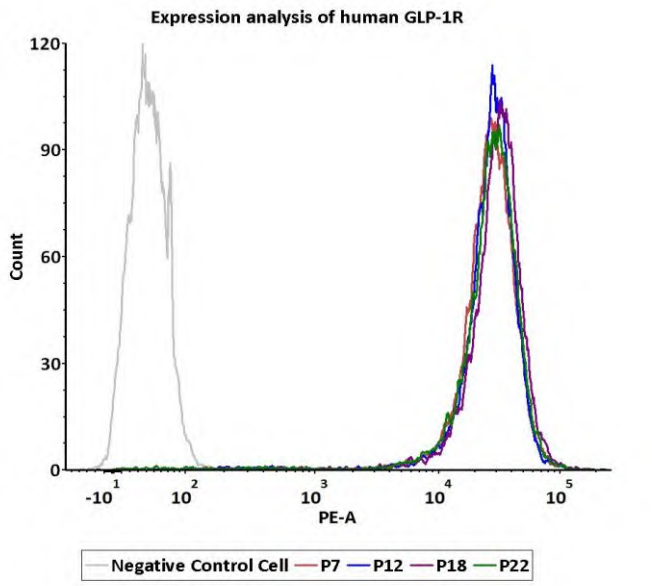
• *Passage Stability*



Passage	MFI for GLP-1R (PE)
P7	26209.5
P12	27283.6
P18	30059.66
P22	27361.13

Fig3. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human GLP-1R on HEK293/Human GLP-1R Stable Cell Line (High Expression) demonstrates consistent mean fluorescent intensity across passage 7-22.

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• *Related Products*

Products	Cat.No.
Human GLP-1R (Luc) HEK293 Reporter Cell	CHEK-ATF096
Human GCGR (Luc) HEK293 Reporter Cell	CHEK-ATF103
Human GIPR (Luc) HEK293 Reporter Cell	CHEK-ATF104
HEK293/Human GLP-1R Stable Cell Line (Medium Expression)	CHEK-ATP161
HEK293/Human GLP-1R Stable Cell Line (Low Expression)	CHEK-ATP162
Human FGF-21 (Luc) HEK293 Reporter Cell	CHEK-ATF163
Human Activin RII (Luc) HEK293 Reporter Cell	CHEK-ATF164
HEK293/Human GPR75 Stable Cell Line	CHEK-ATP174
Human THRA (Luc) HEK293 Reporter Cell	CHEK-ATF180
Human THRB (Luc) HEK293 Reporter Cell	CHEK-ATF181
HEK293/Human GLP-1R&GIPR Stable Cell Line	CHEK-ATP205
HEK293/Human GIPR Stable Cell Line (High Expression)	CHEK-ATP206
HEK293/Human GIPR Stable Cell Line (Medium Expression)	CHEK-ATP207
HEK293/Human GIPR Stable Cell Line (Low Expression)	CHEK-ATP208
HEK293/Human GCGR Stable Cell Line (High Expression)	CHEK-ATP209
HEK293/Human GCGR Stable Cell Line (Medium Expression)	CHEK-ATP210
HEK293/Human GCGR Stable Cell Line (Low Expression)	CHEK-ATP211
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human LDL R Stable Cell Line	CHEK-ATP158