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

HEK293/Human GLP-1R Stable Cell Line (Medium Expression)

Catalog No.	Size
CHEK-ATP161	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 (Gene ID: 2740), 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 (Medium Expression)
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Hygromycin (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 (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Hygromycin B (Invitrogen, Cat.No.10687010)
- 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 (20μg/mL), 1%P/S
- 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_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

• *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℃ with 5% CO_2 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℃ with 5% CO_2 incubator.

Subcultivation Ratio: A subcultivation ratio of 1:6 to 1:10 is recommended.

Medium Renewal: Every 2 to 3 days.

Note: After recovery for 1-2 generations with the complete growth medium not containing the selection marker, if the cell state is well, changing to the culture medium containing the selection marker.

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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 5×10^6 to 1×10^7 cells/mL.
6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transferring to liquid nitrogen storage.

• *Storage*

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

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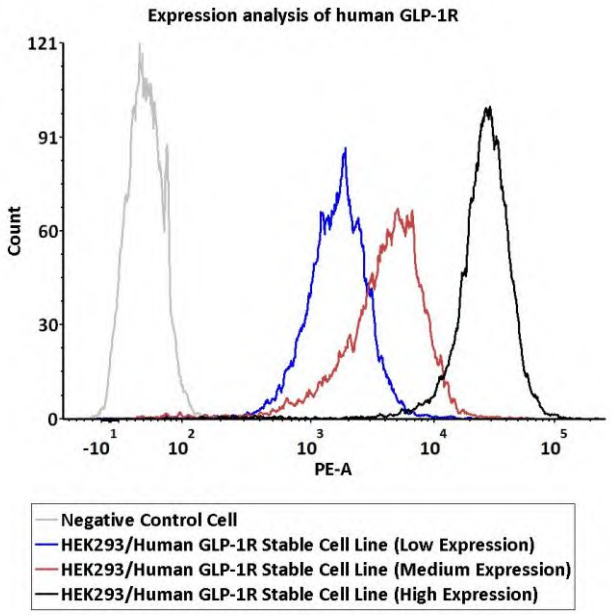
- ***Receptor Assay***



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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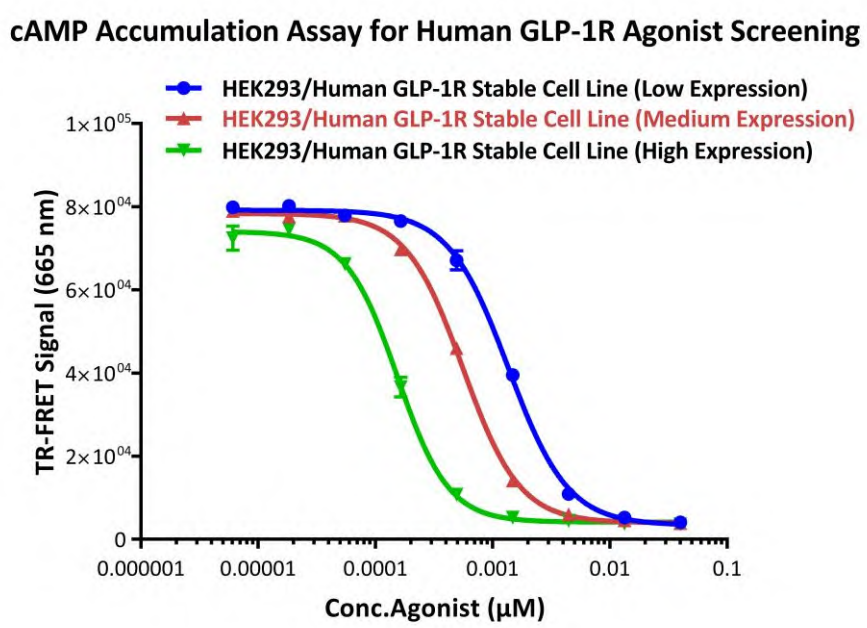
- ***Application***



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 (Medium Expression) was approximately 0.0005579 μM.

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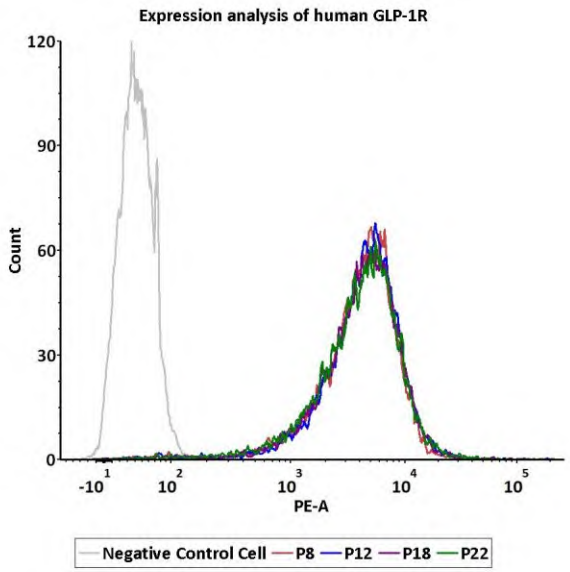
• *Passage Stability*



Passage	MFI for GLP-1R (PE)
P8	4239.74
P12	4409.26
P18	4292.13
P22	4221.43

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 (Medium Expression) demonstrates consistent mean fluorescent intensity across passage 8-22.

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• *Related Products*

Products	**Cat.No.**
HEK293/Human GLP-1R Stable Cell Line (High Expression)	CHEK-ATP160
HEK293/Human GLP-1R Stable Cell Line (Low Expression)	CHEK-ATP162