

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

| Catalog No. | Size   |
|-------------|--|
| CHEK-ATP161 | $2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$ |

### • 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 <sub>2</sub>                             |  |
| Doubling Time          | 22-24 hours  |  |
| Transduction Technique | Lentivirus   |  |



### • Materials Required for Cell Culture

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Hygromycin (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<sub>2</sub> 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°C with 5% CO<sub>2</sub> 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°C 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.

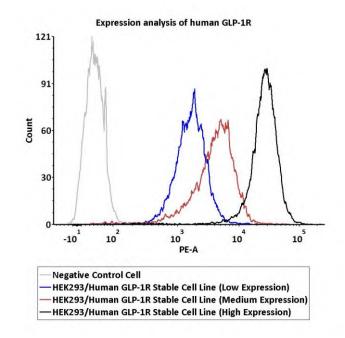


### • Cryopreservation

- 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  $5 \times 10^6$  to  $1 \times 10^7$  cells/mL.
- 6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in  $a 80^{\circ}$ C freezer overnight, then transferring to liquid nitrogen storage.
- Storage
  - **Product format:** Frozen
  - Storage conditions: Liquid nitrogen immediately upon receipt



### • Receptor Assay

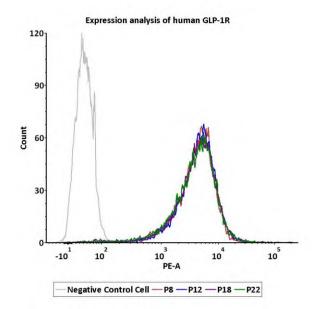


| Catalog No. | Stable Cell Line   | MFI for GLP-1R (PE) |
|-------------|--|---------------------|
| CHEK-ATP162 | HEK293/Human GLP-1R Stable Cell Line (Low Expression)    | 1604.76             |
| CHEK-ATP161 | HEK293/Human GLP-1R Stable Cell Line (Medium Expression) | 4208.08             |
| CHEK-ATP160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



### • Passage Stability



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

**Fig2.** 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.



#### • License Disclosure

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### • Related Products

Products\_ HEK293/Human GLP-1R Stable Cell Line (High Expression) HEK293/Human GLP-1R Stable Cell Line (Low Expression) <u>Cat.No.</u>

CHEK-ATP160 CHEK-ATP162