

# HEK293/Human MC4R Stable Cell Line Data Sheet

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# HEK293/Human MC4R Stable Cell Line Data Sheet

## HEK293/Human MC4R Stable Cell Line

| Catalog No. | Size                                       |
|-------------|--------------------------------------------|
| CHEK-ATP332 | 1 vial contains $\sim 5 \times 10^6$ cells |

### • *Description*

The HEK293/Human MC4R Stable Cell Line was engineered to express the receptor full length human MC4R (Uniprot: P32245). When stimulated with MC4R agonists or  $\alpha$ -MSH, receptor-mediated signaling can induce intracellular cAMP accumulation.

### • *Application*

- Screen for human MC4R agonists based on cAMP accumulation assay

### • *Cell Line Profile*

|                        |                                    |
|------------------------|------------------------------------|
| Cell line              | HEK293/Human MC4R Stable Cell Line |
| Host Cell              | HEK293                             |
| Property               | Adherent                           |
| Complete Growth Medium | DMEM + 10% FBS                     |
| Selection Marker       | Hygromycin B (20 $\mu$ g/mL)       |
| Incubation             | 37°C with 5% CO <sub>2</sub>       |
| 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), 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)
- CO<sub>2</sub> 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<sub>2</sub> 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% CO<sub>2</sub> 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 MC4R 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  $5 \times 10^6$  to  $1 \times 10^7$  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.

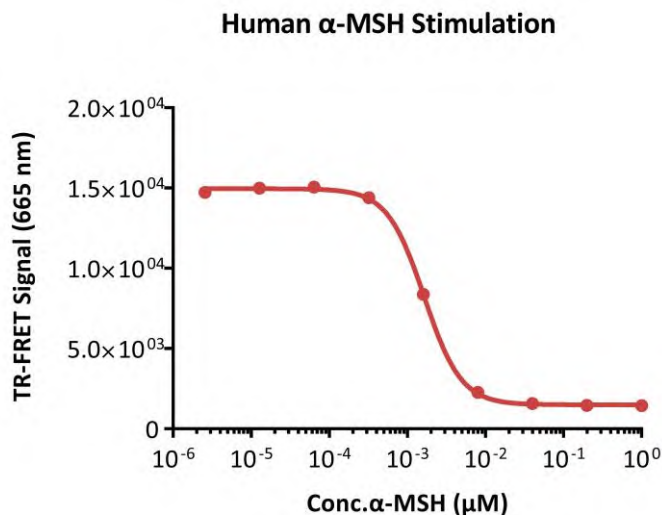
**Note:** It is recommended to establish a cell bank at the earliest possible passage for long-term use.

## • *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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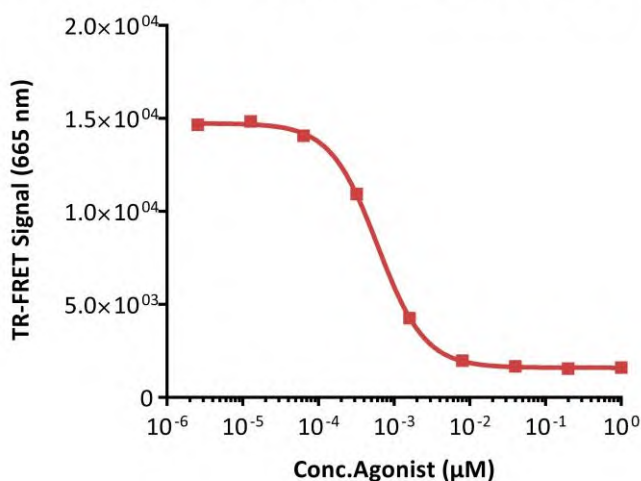
## • Signaling Bioassay



**Fig1. Response to human  $\alpha$ -MSH measured by cAMP accumulation assay.** HEK293/Human MC4R Stable Cell Line were stimulated with serial dilutions of  $\alpha$ -MSH. The EC<sub>50</sub> of  $\alpha$ -MSH was approximately 0.001645  $\mu$ M.

## • Application

### cAMP Accumulation Assay for Human MC4R Agonist Screening



**Fig2. cAMP accumulation assay for human MC4R agonist screening.** HEK293/Human MC4R Stable Cell Line were stimulated with serial dilutions of human MC4R agonist. The EC<sub>50</sub> of human MC4R agonist (Bremelanotide) was approximately 0.0006056  $\mu$ M.

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## • *Related Products*

| <b><u>Products</u></b>                                                 | <b><u>Cat. No.</u></b> |
|------------------------------------------------------------------------|------------------------|
| 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 (High Expression)                 | CHEK-ATP160            |
| 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 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            |
| Human CTR (Luc) BHK 21 Reporter Cell Development Service               | SCBHK-ATF230           |
| Human AMY3R (CTR&RAMP3) (Luc) BHK 21 Reporter Cell Development Service | SCBHK-ATF231           |
| Human APJ R (Luc) HEK293 Reporter Cell                                 | CHEK-ATF242            |
| Human GLP-1R (GFP) HEK293 Reporter Cell                                | CHEK-ATF251            |
| HEK293/Human ASGR2 Stable Cell Line                                    | CHEK-ATP256            |
| Human GIPR (GFP) HEK293 Reporter Cell                                  | CHEK-ATF268            |
| HEK293/Human NPR1 Stable Cell Line                                     | CHEK-ATP245            |
| CHO/Human NPR1 Stable Cell Line                                        | CCHO-ATP290            |