Genomeditech (Shanghai) Co.,Ltd.
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# Product Sheet

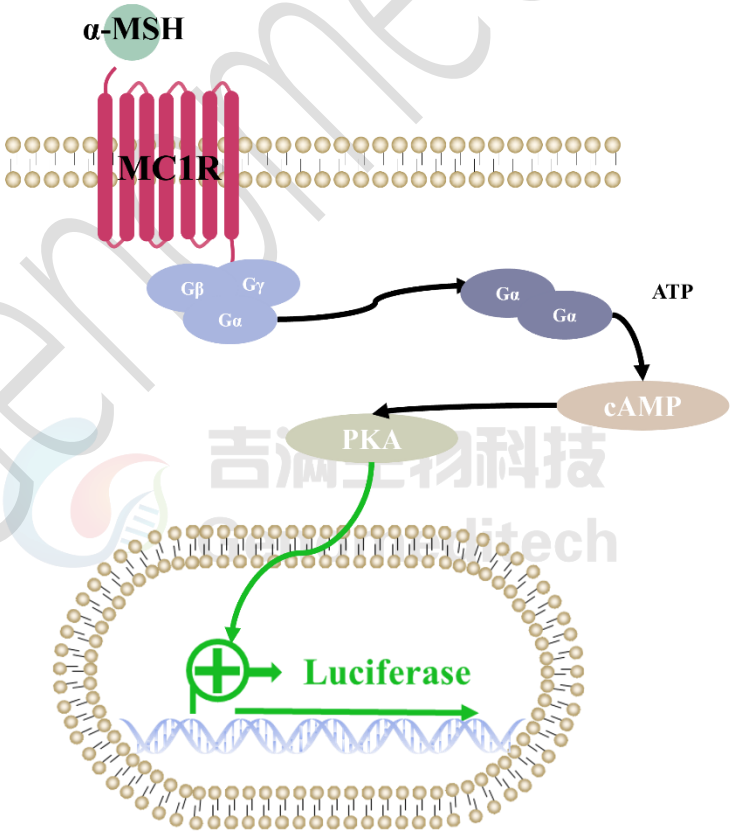
## H_MC1R Reporter CHO-K1 Cell Line

Catalog number: GM-C39435

Version 3.3.1.250704

The MC1R protein is a transmembrane G protein-coupled receptor encoded by the MC1R gene, primarily expressed in melanocytes, keratinocytes, and neurons of the central nervous system. Its core function is to regulate melanin synthesis, UV protection, and inflammatory responses by activating the downstream cAMP signaling pathway upon binding to ligands (such as α-melanocyte-stimulating hormone, α-MSH). Additionally, the MC1R protein is involved in the regulation of energy metabolism, fat distribution, and neurodegenerative diseases (such as Parkinson's disease) and is closely associated with the development and progression of inflammatory diseases (such as psoriasis). In recent years, it has emerged as a potential molecular target for the treatment of skin cancer, obesity, and related conditions.

H_MC1R Reporter CHO-K1 Cell Line is a clonal stable cell line constructed using lentiviral technology, constitutive expression of the MC1R gene, along with signal-dependent expression of a luciferase reporter gene. When α-MSH binds to MC1R, it activates downstream signaling pathways, leading to the expression of luciferase. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of related drugs of MC1R.



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## Specifications

| | |
|---|---|
| **Quantity** | 5E6 Cells per vial,1 mL |
| **Product Format** | 1 vial of frozen cells |
| **Shipping** | Shipped on dry ice |
| **Storage Conditions** | Liquid nitrogen immediately upon receipt |
| **Recovery Medium** | F12K+10% FBS+1% P.S |
| **Growth medium** | F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+4 μg/mL Puromycin |
| **Note** | None |
| **Freezing Medium** | 90% FBS+10% DMSO |
| **Growth properties** | Adherent |
| **Growth Conditions** | 37°C, 5% $CO_2$ |
| **Mycoplasma Testing** | The cell line has been screened to confirm the absence of Mycoplasma species. |
| **Safety considerations** | Biosafety Level 2 |
| **Note** | It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use. |

## Materials

| Reagent | Manufacturer/Catalogue No. |
|---|---|
| F12K | BOSTER/PYG0036 |
| Fetal Bovine Serum | ExCell/FSP500 |
| Pen/Strep | Thermo/15140-122 |
| Blasticidin | Genomeditech/GM-040404 |
| Puromycin | Genomeditech/GM-040401 |
| α -MSH | RHAWN/R032275 |
| GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit | Genomeditech/GM-040513 |

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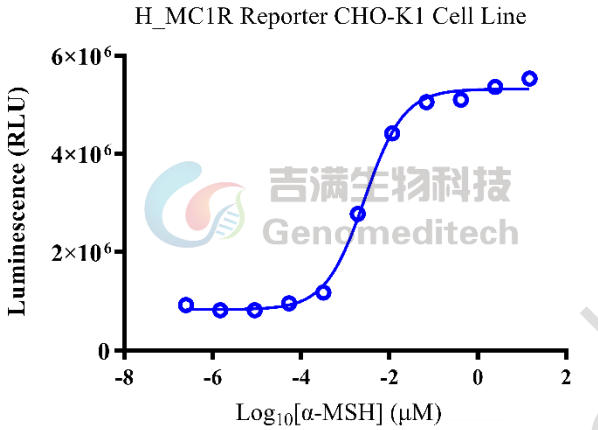
## Figures




| | H_MC1R Reporter CHO-K1 Cell Line |
|---|---|
| EC50 | 0.002749 |

Figure 1 | Response to α-MSH. The H_MC1R Reporter CHO-K1 Cell Line (Cat. GM-C39435) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of α-MSH (RHAWN/R032275) in assay buffer (F12K+1% FBS+1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). The maximum induction fold was approximately [6.6]. Data are shown by drug molar concentration.

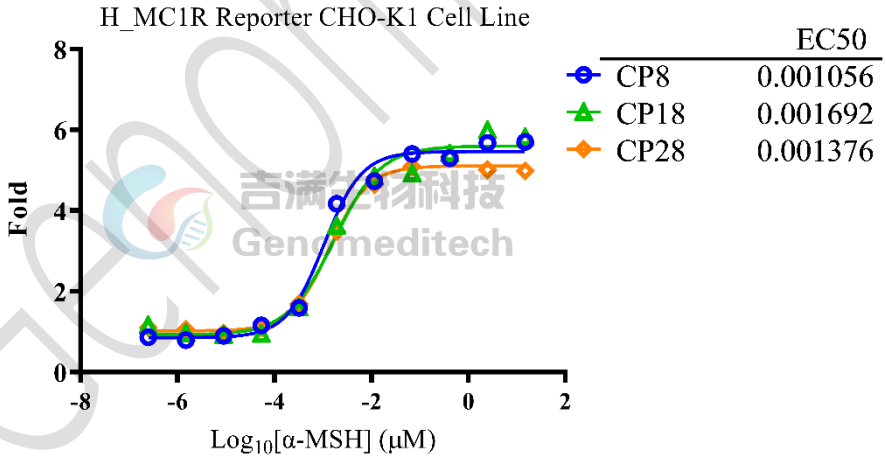



Figure 2 | The passage stability of response to α-MSH. The passage 8, 18 and 28 of H_MC1R Reporter CHO-K1 Cell Line (Cat. GM-C39435) at a concentration of 1E4 cells/well (96-well format) was stimulated with serial dilutions of α-MSH (RHAWN/R032275) in assay buffer (F12K + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit (Cat. GM-040513). Data are shown by drug mass concentration.

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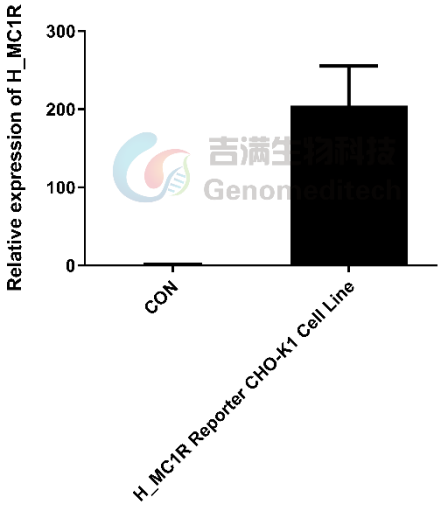


Figure 3 | The mRNA expression levels of H_MC1R Reporter CHO-K1 Cell Line (Cat. GM-C39435) were determined by RT-qPCR.

## Cell Recovery

Recovery Medium: F12K+10% FBS+1% P.S

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).

b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium and spin at approximately 176 x g for 5 minutes. Discard supernatant.

d) Resuspend cell pellet with the recommended recovery medium. And dispense into appropriate culture dishes.

e) Incubate the culture at 37°C in a suitable incubator. A 5% $CO_2$ in air atmosphere is recommended if using the medium described on this product sheet.

## Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

a) Centrifuge at 176 x g for 3 minutes to collect cells.

b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.

c) Aliquot 1 mL into each vial.

d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

## Cell passage

Growth medium: F12K+10% FBS+1% P.S+4 μg/mL Blasticidin+4 μg/mL Puromycin

For the first 1 to 2 passages post-resuscitation, use the recovery medium. Once the cells have stabilized, switch to a growth medium.

a) Remove and discard culture medium.

b) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.

c) Add 1.0 mL of 0.25% (w/v) Trypsin-EDTA solution to dish and observe cells under an inverted microscope until cell layer is dispersed (usually within 2 to 3 minutes at 37°C).

d) Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

e) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.

f) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.

g) Incubate cultures at 37°C.

**Subcultivation Ratio: A subcultivation ratio of 1:4 - 1:5 is recommended**

**Medium Renewal: Every 2 to 3 days**

## Notes

a) After the stabilization of the cell condition, there will be fewer dead cells post-passage, the cell growth rate will tend to stabilize, cell morphology will become uniform, and the cells will appear robust.

## License Agreement:

**By purchasing and using this cell line product, the user voluntarily agrees to accept and abide by the following policies:**

- This cell line product is restricted to research use only and shall not be used for any commercial purposes.
- This product is strictly prohibited from being used in the diagnosis or treatment of human or animal diseases, and shall not be directly used in experiments involving humans.
- Users are not permitted to modify the cell line in any way, nor to share, distribute, sell, sublicense, or otherwise transfer the licensed materials or their progeny to other laboratories, departments, research institutes, hospitals, universities, biotechnology companies, or any other third parties, except for research activities outsourced on behalf of the licensee.
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