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# Product Sheet

## H_CD4 CCR5 CXCR4 Hela Cell Line

Catalog number: GM-C39495

Version 3.3.1.250813

| | |
|---|---|
| **Description** | H_CD4 CCR5 CXCR4 Hela Cell Line is a clonal stable Hela cell line that constitutively expresses the Human CD4,Human CCR5 and Human CXCR4 genes, constructed using lentiviral technology. |
| **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 |
| **Target** | Human CD4 & Human CCR5 & Human CXCR4 |
| **Gene ID/Uniprot ID** | P01730 & P51681 & P61073-1 |
| **Host Cell** | Hela |
| **Recovery Medium** | DMEM/F12+10% FBS+1% P.S |
| **Growth medium** | DMEM/F12+10% FBS+1% P.S+3 μg/mL Blasticidin+0.5 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. |
|---|---|
| DMEM/F12 | Gibco/C11330500BT |
| Fetal Bovine Serum | ExCell/FSP500 |
| Pen/Strep | Thermo/15140-122 |
| Puromycin | Genomeditech/GM-040401 |
| Blasticidin | Genomeditech/GM-040404 |
| Anti-CCR5 hIgG4 Antibody(leronlimab) | Genomeditech/GM-87687AB |
| Anti-H_CXCR4 hIgG4 Antibody (Ulocuplumab) | Genomeditech/GM-27445AB |
| Anti-H_CD4 hIgG1 Antibody(Tregalizumab) | Genomeditech/GM-28752AB |

## Figures

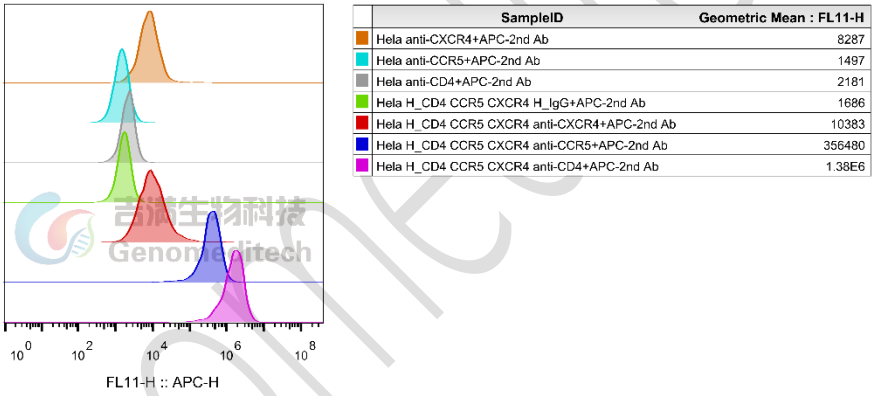


Figure 1 | H_CD4 CCR5 CXCR4 Hela Cell Line (Cat. GM-C39495) was determined by flow cytometry using AAnti-CCR5 hIgG4 Antibody(leronlimab) (Cat. GM-87687AB),Anti-H_CXCR4 hIgG4 Antibody (Ulocuplumab) (Cat. GM-27445AB)and Anti-H_CD4 hIgG1 Antibody(Tregalizumab)(Cat. GM-28752AB).

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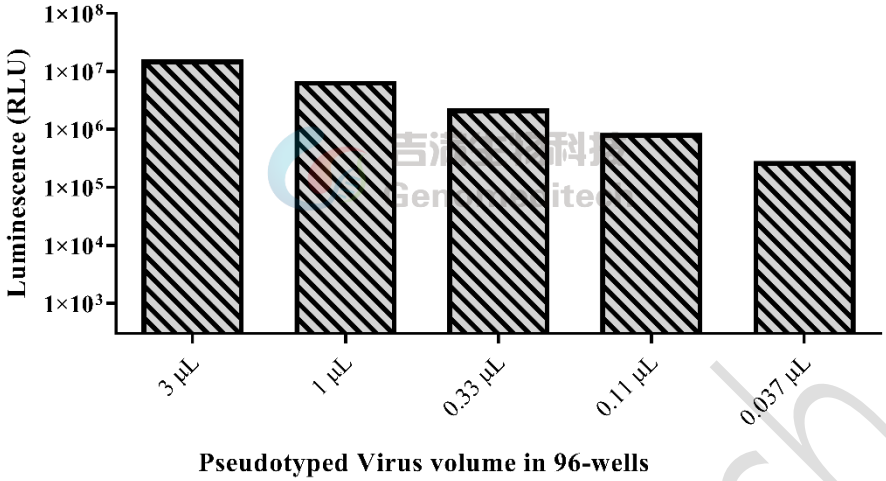


Figure 2 | H_CD4 CCR5 CXCR4 Hela Cell Line (Cat. GM-C39495) at were infected by different amounts of HIV-1 (BG505) Pseudotyped Virus (GFP-Luciferase) (Cat. GM-0220PV196), respectively.

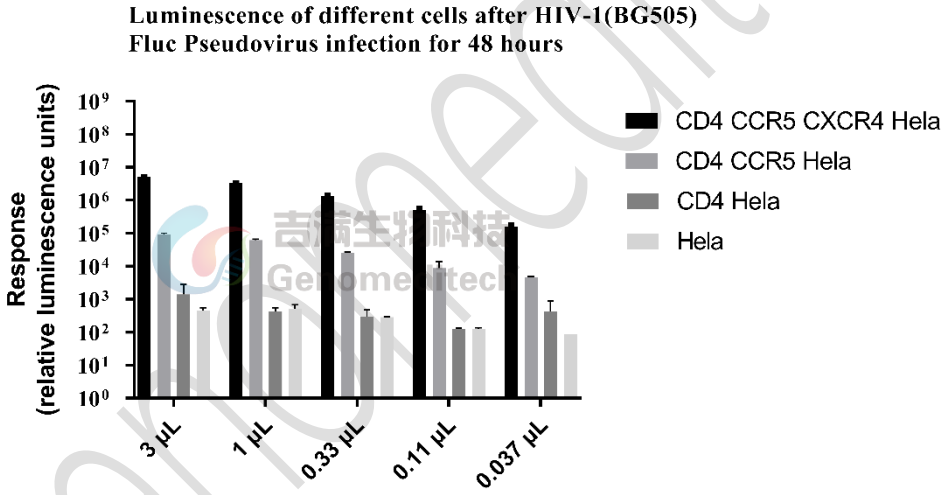


Figure 3 | Luciferase activity was measured after infection with gradient-diluted HIV-1 (BG505) pseudovirus in the following cell lines: H_CD4 CCR5 CXCR4 Hela Cell Line (Cat. GM-C39495), H_CD4 CCR5 HeLa Cell Line, H_CD4 HeLa Cell Line, and HeLa.

## Cell Recovery

Recovery Medium: DMEM/F12+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: DMEM/F12+10% FBS+1% P.S+3 μg/mL Blasticidin+0.5 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) Subculturing is necessary when the cell density reaches 80%. It is recommended to perform subculturing at a ratio of 1:2 to 1:3 every 2 to 3 days.
b) Remove and discard culture medium.
c) Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
d) 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).
e) 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.
f) Add 2.0 mL of growth medium to mix well and aspirate cells by gently pipetting.
g) After centrifugation, resuspend the pellet and add appropriate aliquots of the cell suspension to new culture vessels.
h) Incubate cultures at 37°C.

**Subcultivation Ratio: A subcultivation ratio of 1:2 - 1:3 is recommended**

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

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## Notes

a) Fetal bovine serum (FBS) needs to be heat-inactivated at 56°C for 30 minutes, which can inactivate complement and certain viruses without significantly affecting the activity of most growth factors and cytokines.

## 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.
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