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# Product Sheet

## H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line

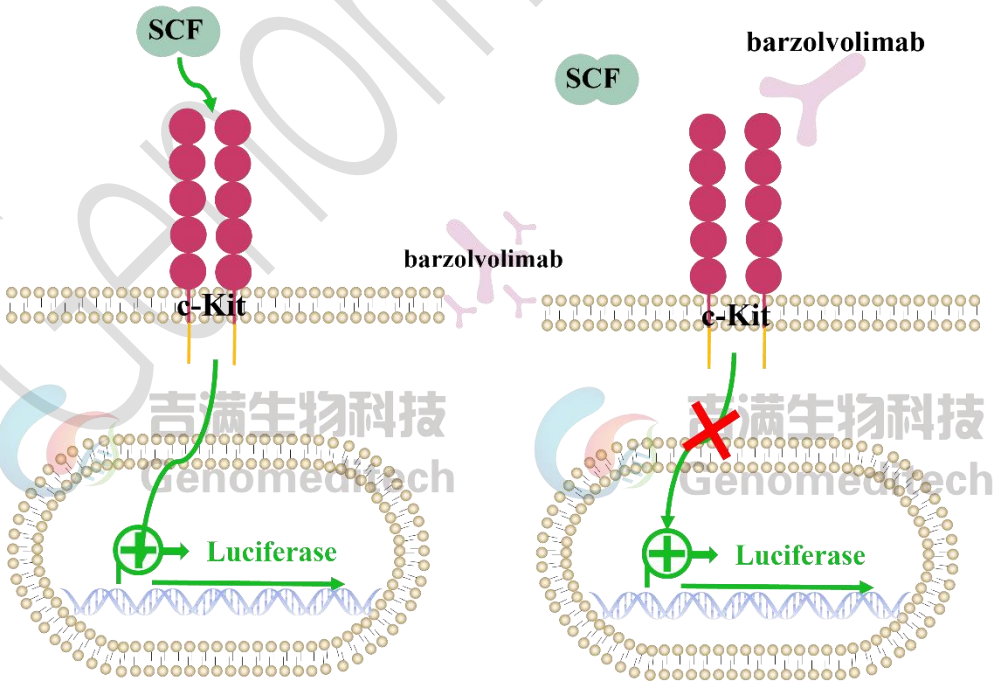
Catalog number: GM-C37519

Version 3.3.1.250103

c-kit (CD117) is a tyrosine kinase receptor encoded by the KIT gene, mainly found in hematopoietic and mesenchymal stem cells, as well as some tumors. It is crucial for embryonic development and the proliferation and differentiation of melanocytes, mast cells, and germ cells. c-kit activation depends on its ligand, stem cell factor (SCF), which activates downstream pathways that regulate cell survival, proliferation, and migration.

The c-kit signaling pathway is activated when SCF binds to the c-kit, leading to dimerization and autophosphorylation. The Ras-MAPK pathway promotes proliferation through ERK activation, while the PI3K-Akt pathway enhances survival and anti-apoptotic effects. Abnormal c-kit activation is seen in certain tumors, like gastrointestinal stromal tumors, making it a significant therapeutic target.

H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line is a clonal stable HEK-293 cell line constructed using lentiviral technology, constitutively expressing c-Kit(CD117) chimeric receptor, along with signal-dependent expression of a luciferase reporter gene. When SCF binds to c-Kit(CD117), it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can inhibit this signal transmission. The luciferase readout represents the activation level of the signaling pathway and can thus be used for evaluating the in vitro effects of neutralizing antibody of c-Kit(CD117).



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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** | DMEM+10% FBS+1% P.S |
| **Growth medium** | DMEM+10% FBS+1% P.S+4 μg/mL Blasticidin+0.75 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 | Gibco/C11995500BT |
| Fetal Bovine Serum | Cegrogen biotech/A0500-3010 |
| Pen/Strep | Thermo/15140-122 |
| Blasticidin | Genomeditech/GM-040404 |
| Puromycin | Genomeditech/GM-040401 |
| Human SCF Protein; His Tag | Genomeditech/GM-87651RP |
| Anti-c-Kit(CD117) hIgG1 Antibody(barzolvolimab) | Genomeditech/GM-86542AB |
| GMOne-Step Luciferase Reporter Gene Assay Kit | Genomeditech/GM-040503 |

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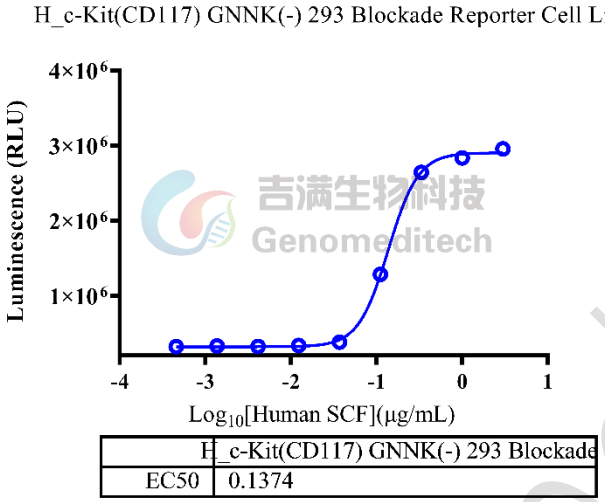
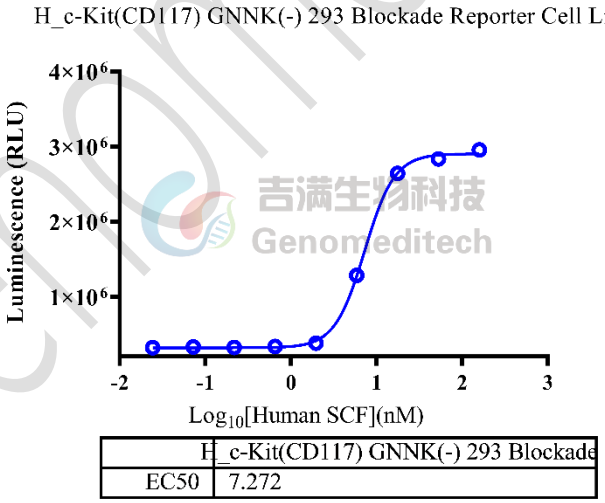
## Figures



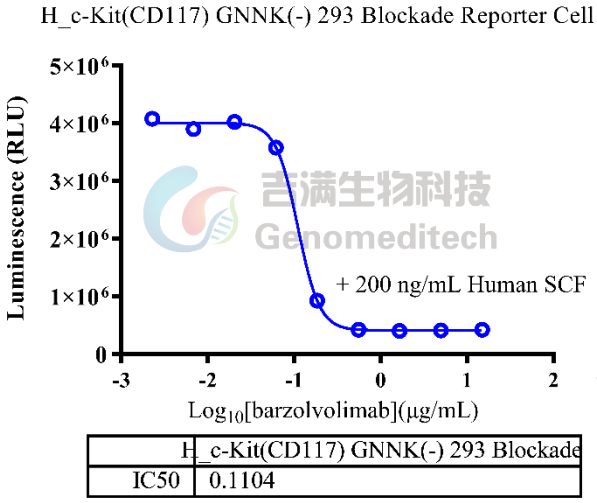

| | H_c-Kit(CD117) GNNK(-) 293 Blockade |
|---|---|
| EC50 | 0.1374 |

Figure 1 | Response to Human SCF Protein; His Tag. H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line (Cat. GM-C37519) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human SCF Protein (Cat. GM-87651RP) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [8.5]. Data are shown by drug mass concentration.



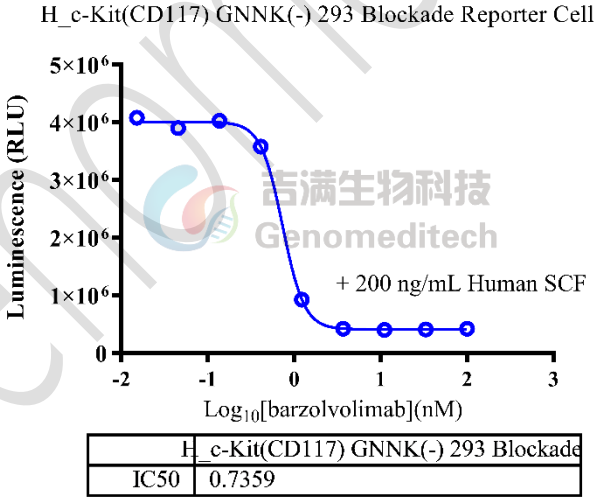

| | H_c-Kit(CD117) GNNK(-) 293 Blockade |
|---|---|
| EC50 | 7.272 |

Figure 2 | Response to Human SCF Protein; His Tag. H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line (Cat. GM-C37519) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human SCF Protein (Cat. GM-87651RP) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [8.5]. Data are shown by drug molar concentration.




| | H_c-Kit(CD117) GNNK(-) 293 Blockade |
|---|---|
| IC50 | 0.1104 |

Figure 3 | Response to Anti-c-Kit(CD117) hIgG1 Antibody(barzolvolimab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line (Cat. GM-C37519) in a 96-well plate for 1 hour. Subsequently, the Human SCF Protein (Cat.GM-87651RP) at a concentration of 20 ng/well was added, and the coculture proceeded for an additional 16 hours. Firefly luciferase activity is measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [10.6]. Data are shown by drug mass concentration.




| | H_c-Kit(CD117) GNNK(-) 293 Blockade |
|---|---|
| IC50 | 0.7359 |

Figure 4 | Response to Anti-c-Kit(CD117) hIgG1 Antibody(barzolvolimab). Serial dilutions of antibodies were incubated with 1.5E4 cells/well of the H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line (Cat. GM-C37519) in a 96-well plate for 1 hour. Subsequently, the Human SCF Protein (Cat.GM-87651RP) at a concentration of 20 ng/well was added, and the coculture proceeded for an additional 16 hours. Firefly luciferase activity is measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [10.6]. Data are shown by drug molar concentration.

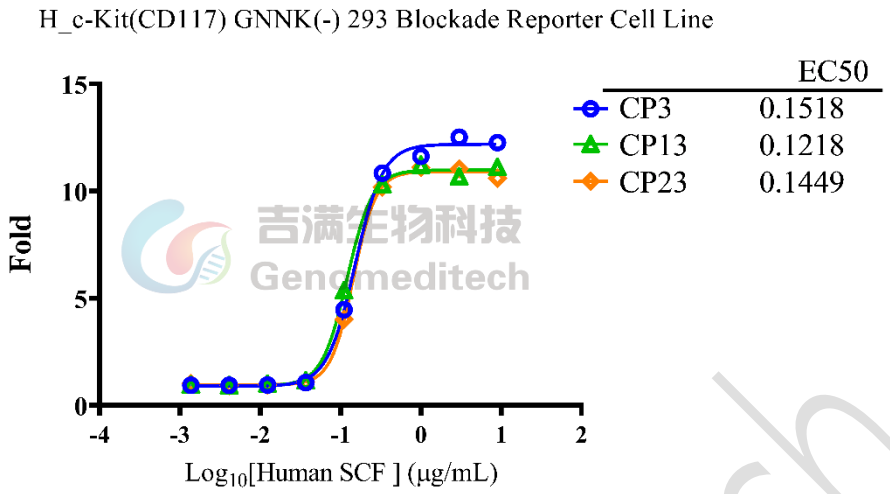



Figure 5 | The passage stability of response to Human SCF Protein. The passage 3, 13, and 23 of H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line (Cat. GM-C37519) at a concentration of 1.5E4 cells/well (96-well format) were stimulated with serial dilutions of Human SCF Protein (Cat. GM-87651RP) in assay buffer (DMEM + 1% FBS + 1% P.S) for 16 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.

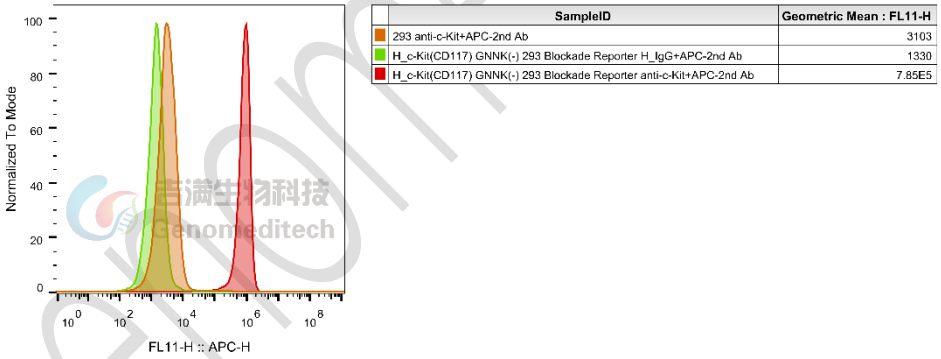



Figure 6 | H_c-Kit(CD117) GNNK(-) 293 Blockade Reporter Cell Line was determined by flow cytometry using Anti-c-Kit(CD117) hIgG1 Antibody(barzolvolimab) (Cat. GM-86542AB).

## Cell Recovery

Recovery Medium: DMEM+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+10% FBS+1% P.S+4 μg/mL Blasticidin+0.75 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 1:3 to 1:4 every 2-3 days. Ensure that the density does not exceed 80%, as overcrowding can lead to reduced viability due to compression.
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 30 to 60 seconds 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 1:3 - 1:4 is recommended**

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

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## Notes

a) Upon initial thawing, a higher number of dead cells is observed, which is a normal phenomenon. Significant improvement is seen after adaptation. Once the cells reach a stable state, the number of dead cells decreases after subculturing and the cell growth rate becomes stable.

b) Ensure that the cell density does not exceed 80%, as overcrowding may lead to reduced viability due to compression.

## Related Products

| c-Kit：SCF | |
|---|---|
| Cynomolgus_c-Kit(CD117) GNNK(-) CHO-K1 Cell Line | H_c-Kit(CD117) GNNK(-) CHO-K1 Cell Line |
| H_c-Kit(CD117) GNNK(-) HEK-293 Cell Line | H_c-Kit(CD117) GNNK(+) CHO-K1 Cell Line |
| Anti-c-Kit(CD117) hIgG1 Antibody(barzolvolimab) | Anti-c-Kit(CD117) hIgG1 Antibody(briquilimab) |
| Anti-c-Kit(CD117) hIgG1 Reference Antibody(barbio) | |
| Biotinylated Human SCF Protein; His-Avi Tag | Cynomolgus c-Kit(CD117) Protein; His Tag |
| Human c-Kit(CD117) Protein; hFc Tag | Human c-Kit(CD117) Protein; His Tag |
| Human SCF Protein; His Tag | Human SCF Protein; mFc Tag |
| MRGPRX2 | |
| H_MRGPRX2 Reporter Cell Line | Cynomolgus_MRGPRX2 CHO-K1 Cell Line |
| Cynomolgus_MRGPRX2 HEK-293 Cell Line | H_MRGPRX2 CHO-K1 Cell Line |
| H_MRGPRX2 HEK-293 Cell Line | |

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