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Product Sheet

IL-4/IL-13 Reporter 293 Cell Line

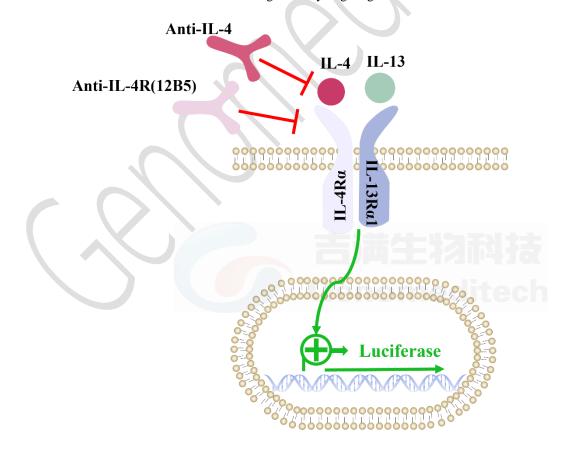
Catalog number: GM-C01511

Version 3.3.1.241218

Interleukin-4 (IL-4) is a cytokine essential for differentiating Th0 cells into anti-inflammatory Th2 cells. Secreted mainly by Th2 cells, IL-4 promotes B cell proliferation and IgE antibody synthesis, crucial in allergies. IL-4 activates downstream signaling pathways through receptor binding.

Similarly, IL-13 is produced by T cells, including Th2 cells, and shares biological traits with IL-4. Its complex signaling involves receptors like IL-13Rα1, activating downstream pathways.

The IL-4/IL-13 Reporter 293 cell line is a clonal stable 293 cell line with signal-dependent expression of a luciferase reporter gene, and it endogenously expresses IL-4Rα and IL-13Rα1. When IL-4/IL-13 binds to the receptor, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can block this signal transmission. The measurement of luciferase activity indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of a neutralizing antibody targeting IL-4 and IL-13.





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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% CO₂

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. |
|---|-----------------------------|
| Puromycin | Genomeditech/GM-040401 |
| Blasticidin | Genomeditech/GM-040404 |
| Pen/Strep | Thermo/15140-122 |
| Fetal Bovine Serum | Cegrogen biotech/A0500-3010 |
| DMEM | Gibco/C11995500BT |
| Recombinant Human IL-4 Protein | R&D SYSTEMS/204-IL/CF |
| Recombinant Human IL-13 Protein | Sino Biological/10369-HNAC |
| Human IL-4 Antibody | R&D SYSTEMS/MAB204 |
| Anti-IL-4R hIgG1 Antibody(12B5) | Genomeditech/GM-46268AB |
| GMOne-Step Luciferase Reporter Gene Assay Kit | Genomeditech/GM-040503 |
| APC Rabbit anti-Human CD213a1/IL-13Rα1 mAb | Abclonal/A26763 |

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Figures

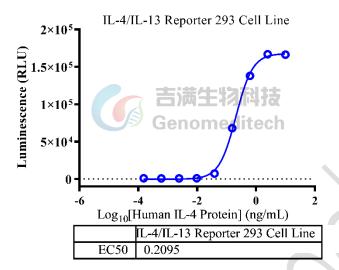


Figure 1 | Response to Recombinant Human IL-4 Protein. The IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a concentration of 2E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-4 Protein (R&D SYSTEMS/204-IL/CF) in assay buffer (DMEM+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [177.9]. Data are shown by drug mass concentration.

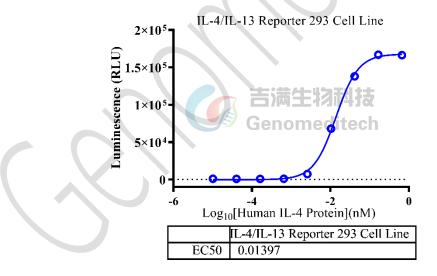


Figure 2 | Response to Recombinant Human IL-4 Protein. The IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a concentration of 2E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-4 Protein (R&D SYSTEMS/204-IL/CF) in assay buffer (DMEM+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [177.9]. Data are shown by drug molar concentration.



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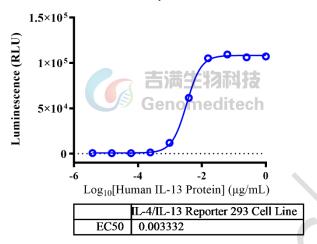


Figure 3 | Response to Recombinant Human IL-13 Protein. The IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a concentration of 2E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-13 Protein (Sino Biological/10369-HNAC) in assay buffer (DMEM+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [117.1]. Data are shown by drug mass concentration.

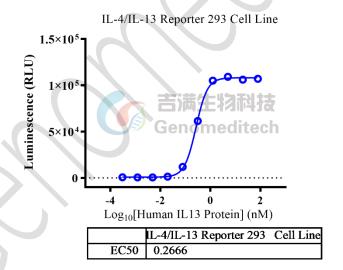
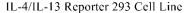


Figure 4 | Response to Recombinant Human IL-13 Protein. The IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a concentration of 2E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-13 Protein (Sino Biological/10369-HNAC) in assay buffer (DMEM+1% FBS+1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [117.1]. Data are shown by drug molar concentration.



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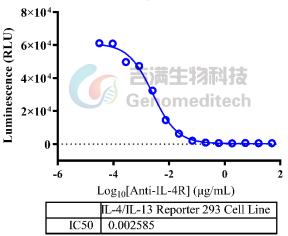


Figure 5 | Response to Anti-IL-4R hIgG1 Antibody(12B5). Serial dilutions of Anti-IL-4R hIgG1 Antibody(12B5)(Cat. GM-46268AB) were incubated with 2E4 cells/well of the IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) in a 96-well plate for 1 hour. Then, 0.2 ng/mL of Recombinant Human IL-4 Protein (R&D SYSTEMS/204-IL/CF) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 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 [122.2]. Data are shown by drug mass concentration.

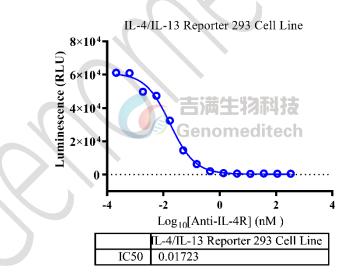


Figure 6 | Response to Anti-IL-4R hIgG1 Antibody(12B5). Serial dilutions of Anti-IL-4R hIgG1 Antibody(12B5)(Cat. GM-46268AB) were incubated with 2E4 cells/well of the IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) in a 96-well plate for 1 hour. Then, 0.2 ng/mL of Recombinant Human IL-4 Protein (R&D SYSTEMS/204-IL/CF) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 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 [122.2]. Data are shown by drug molar concentration.



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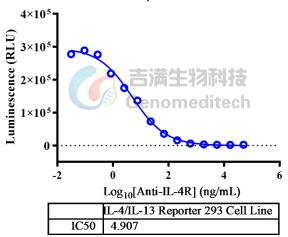


Figure 7 | Response to Anti-IL-4R hIgG1 Antibody(12B5). Serial dilutions of Anti-IL-4R hIgG1 Antibody(12B5)(Cat. GM-46268AB) were incubated with 2E4 cells/well of the IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) in a 96-well plate for 1 hour. Then, 3.3 ng/mL of Recombinant Human IL-13 Protein (Sino Biological/10369-HNAC) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 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 [130.0]. Data are shown by drug mass concentration.

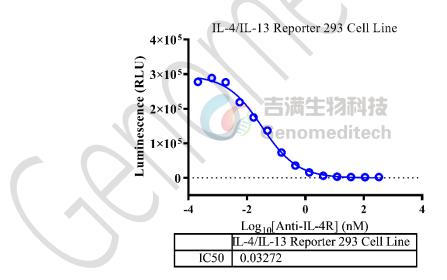


Figure 8 | Response to Anti-IL-4R hIgG1 Antibody(12B5). Serial dilutions of Anti-IL-4R hIgG1 Antibody(12B5)(Cat. GM-46268AB) were incubated with 2E4 cells/well of the IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) in a 96-well plate for 1 hour. Then, 3.3 ng/mL of Recombinant Human IL-13 Protein (Sino Biological/10369-HNAC) was added, and the incubation continued in assay buffer (DMEM + 1% FBS + 1% P.S) for 7 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 [130.0]. Data are shown by drug molar concentration.



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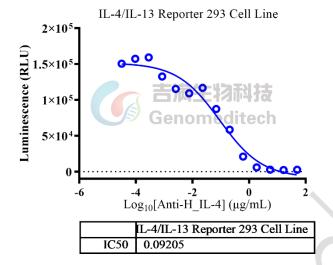


Figure 9 | Response to Human IL-4 Antibody. Begin by preparing the IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a density of 1.5E4 cells/well in a 96-well format. The serial dilutions of Human IL-4 Antibody (R&D SYSTEMS/MAB204) were incubated with 0.2 ng/mL of Recombinant Human IL-4 Protein (R&D SYSTEMS/204-IL/CF) for 1 hour. After pre-incubation, the mixture was added to the IL-4/IL-13 Reporter 293 Cell Line and incubated for 6 hours in assay buffer (DMEM+1% FBS+1% P.S). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [66.3]. Data are shown by drug mass concentration.

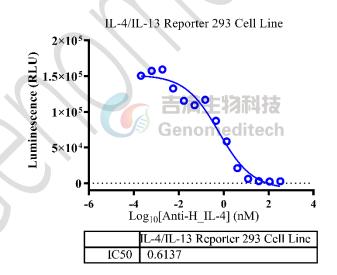


Figure 10 | Response to Human IL-4 Antibody. Begin by preparing the IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a density of 1.5E4 cells/well in a 96-well format. The serial dilutions of Human IL-4 Antibody (R&D SYSTEMS/MAB204) were incubated with 0.2 ng/mL of Recombinant Human IL-4 Protein (R&D SYSTEMS/204-IL/CF) for 1 hour. After pre-incubation, the mixture was added to the IL-4/IL-13 Reporter 293 Cell Line and incubated for 6 hours in assay buffer (DMEM+1% FBS+1% P.S). Firefly luciferase activity is then measured using

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the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated a maximum blocking fold of approximately [66.3]. Data are shown by drug molar concentration.

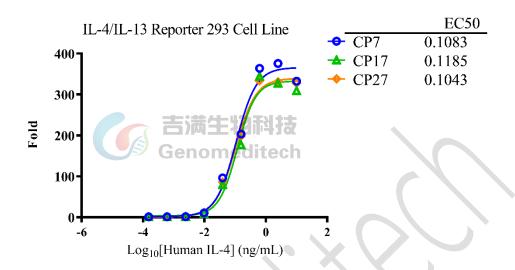


Figure 11 | Response to Recombinant Human IL-4 Protein. The passage 7,17 and 27 of IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-4 Protein (R&D SYSTEMS/204-IL/CF) in assay buffer (DMEM+1% FBS+1% P.S) for 7 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.

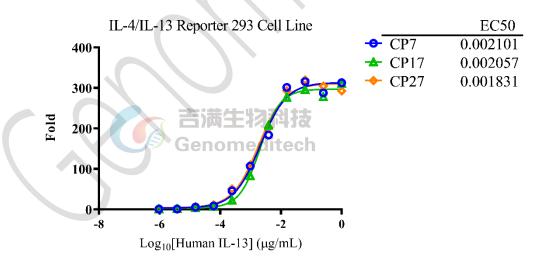


Figure 12 | Response to Recombinant Human IL-13 Protein. The passage 7,17 and 27 of IL-4/IL-13 Reporter 293 Cell Line (Cat. GM-C01511) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Human IL-13 Protein (Sino Biological/10369-HNAC) in assay buffer (DMEM+1% FBS+1% P.S) for 7 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.

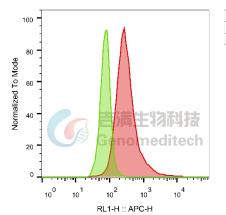


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| SampleID | Geometric Mean : RL1-H |
|---|------------------------|
| IL-4 IL-13 Reporter 293 H_IgG+APC-2nd Ab | 76.4 |
| IL-4 IL-13 Reporter 293 anti-IL-4R+APC-2nd Ab | 318 |
| | |

Figure 13 | IL-4/IL-13 Reporter 293 Cell Line was determined by flow cytometry using Anti-IL-4R hIgG1 Antibody(12B5) (Cat. GM-23373AB).

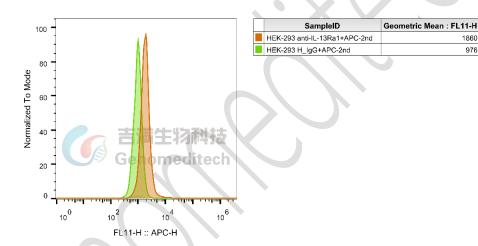


Figure 14 | HEK-293 Cell Line was determined by flow cytometry using APC Rabbit anti-Human CD213a1/IL-13Rα1 mAb (ABclonal/A26763).

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.

- 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.

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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% CO₂ 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

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.



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Related Products

| Related 1 founcts | | | | |
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| H_OX40 CHO-K1 Cell Line | H_OX40L CHO-K1 Cell Line | | | |
| H_OX40L HEK-293 Cell Line | | | | |
| Anti-H_OX40 hIgG2 Antibody(Ivuxolimab) | Anti-OX40L hIgG1 Reference Antibody(Oxebio) | | | |
| Anti-OX40L hIgG4 Antibody(Amlitelimab) | Anti-OX40L hIgG4 Reference Antibody(Amlbio) | | | |
| Biotinylated Human OX40L Protein; His-Avi Tag | Cynomolgus OX40 Protein; His Tag | | | |
| Cynomolgus OX40L Protein; His Tag | Cynomolgus OX40L Protein; mFc Tag | | | |
| Human OX40 Protein; His Tag | Human OX40L Protein; His Tag | | | |
| Human OX40L Protein; mFc Tag | | | | |
| IL-4/IL-13 | | | | |
| IL-4 Reporter Cell Line | IL-4/IL-13 Reporter 293 DDX35TM Cell Line | | | |
| Cynomolgus_IL4R CHO-K1 Cell Line | H_IL4R CHO-K1 Cell Line | | | |
| Anti-IL-4R hIgG1 Antibody(12B5) | Anti-IL4R hIgG4 Antibody(Dupilumab) | | | |
| Anti-IL4R hIgG4 Reference Antibody (Dupbio) | | | | |
| Human IL-4R alpha Protein; mFc Tag | | | | |
| IL-31 | | | | |
| H_IL-31 Reporter Cell Line | Cynomolgus_IL31RA CHO-K1 Cell Line | | | |
| H_IL31RA CHO-K1 Cell Line | H_IL31RA HEK-293 Cell Line | | | |
| H_IL-31RA OSMR Baf3 Cell Line | | | | |
| Anti-IL31 hIgG1 Antibody(mAb33) | Anti-IL31RA hIgG1 Antibody(NA633) | | | |
| Anti-IL31RA hIgG2 Antibody(Nemolizumab) | Anti-OSMR hIgG4 Antibody(Vixarelimab) | | | |
| TSLP:TSLPR | | | | |
| H_TSLP Reporter Cell Line | H_TSLPR CHO-K1 Cell Line | | | |
| Anti-H_TSLPR hIgG1 Antibody | Anti-TSLP hIgG2 Reference Antibody(Tezbio) | | | |
| Anti-TSLP hIgG2 Antibody(Tezepelumab) | | | | |
| Cynomolgus TSLP Protein; His Tag | Human TSLP Protein; His Tag | | | |
| | IL-5 | | | |
| H_IL-5 Reporter 293 Cell Line | H_IL-5RA CHO-K1 Cell Line | | | |
| H_IL-5RA HEK-293 Cell Line | | | | |
| Anti-IL5 hIgG4 Antibody(Reslizumab) | Anti-IL-5R hIgG1 Antibody(Benralizumab) | | | |
| MI | RGPRX2 | | | |
| H_MRGPRX2 Reporter Cell Line | Cynomolgus_MRGPRX2 CHO-K1 Cell Line | | | |
| H_MRGPRX2 CHO-K1 Cell Line | H_MRGPRX2 HEK-293 Cell Line | | | |
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