

## 产品手册

### H\_BCMA Reporter 293 Cell Line

### H\_BCMA Reporter 293 细胞系

For research use only!

本品仅供科研使用，严禁用于治疗！

版本号：V2.12.1

## 目录

|         |                                           |    |
|---------|-------------------------------------------|----|
| 一、      | 产品基本信息及组分 .....                           | 3  |
| 二、      | 包装、运输及储存 .....                            | 3  |
| 三、      | 产品描述 .....                                | 4  |
| 四、      | 材料准备 .....                                | 5  |
| 1.      | 细胞培养、冻存、复苏试剂准备 .....                      | 5  |
| 2.      | 试剂耗材准备 .....                              | 5  |
| 五、      | 细胞复苏、传代、冻存 .....                          | 6  |
| 1.      | 细胞复苏 .....                                | 6  |
| 2.      | 细胞传代（以 10 cm 皿为例） .....                   | 6  |
| 3.      | 细胞冻存 .....                                | 6  |
| 六、      | 使用方法（示例） .....                            | 7  |
| 1.      | 配体激活验证实验——BAFF .....                      | 7  |
| 1)      | 加样步骤 .....                                | 7  |
| 2)      | 报告基因检测 .....                              | 8  |
| 3)      | 验证结果 .....                                | 8  |
| 2.      | BAFF 激活，抗体 block 实验 .....                 | 9  |
| 1)      | 加样步骤 .....                                | 9  |
| 2)      | 报告基因检测 .....                              | 10 |
| 3)      | 验证结果 .....                                | 11 |
| 3.      | 共培养激活后，Anti-APRIL 抗体 block 实验 .....       | 11 |
| 1)      | 加样步骤 .....                                | 12 |
| 2)      | 报告基因检测 .....                              | 13 |
| 3)      | 验证结果 .....                                | 13 |
| 附录 1    | BAFF Protein 激活，Anti-BAFF 抗体 block .....  | 14 |
| 附录 2    | APRIL Protein 激活结果 .....                  | 14 |
| 附录 3    | BAFF Protein 激活后，Anti-BCMA 抗体 block ..... | 15 |
| 附录 4    | 共培养激活结果 .....                             | 15 |
| 附录 5    | 共培养激活后，使用 Anti-BCMA 抗体 block .....        | 16 |
| 附录 6    | 流式验证结果 .....                              | 17 |
| 相关产品    | .....                                     | 17 |
| 使用许可协议： | .....                                     | 18 |

## 一、 产品基本信息及组分

### 基本信息

| 产品编号      | 产品名称                          | 规格           |
|-----------|-------------------------------|--------------|
| GM-C44705 | H_BCMA Reporter 293 Cell Line | 5E6 Cells/mL |

### 组成成分

| 产品编号      | 产品名称                          | 规格           | 数量  | 储存     |
|-----------|-------------------------------|--------------|-----|--------|
| GM-C44705 | H_BCMA Reporter 293 Cell Line | 5E6 Cells/mL | 1 管 | -196°C |

## 二、 包装、运输及储存

1. 细胞系产品干冰运输，-196°C 以下（冰箱或液氮的气相）长期储存。
2. 接触产品请带手套。请收到产品立即确认产品是否为冻存状态，-196°C 以下（冰箱或液氮的气相）长期储存。
3. 本产品相关 Assay，应在二级生物安全实验室或生物安全柜中进行。

### 三、 产品描述

B 细胞成熟蛋白 (BCMA、CD269、TNFRSF17) 是 TNF 受体超家族的成员之一。BCMA 在免疫器官和成熟 B 淋巴细胞中表达。对 B 细胞发育和自身免疫反应很重要。BCMA 被其配体激活, 可促进 B 细胞的分化和增殖。

BCMA 的配体是由巨噬细胞和树突状细胞产生的 BAFF (b 细胞激活因子) 和 APRIL (增殖诱导配体), BAFF 和 APRIL 具有可溶性同三聚体的生物活性。

在多发性骨髓瘤中, BCMA 在恶性浆细胞上以较高水平广泛表达, 并且 BCMA 表达随着从正常细胞向活性多发性骨髓瘤的进展而增加。因此 BCMA 是较好的肿瘤靶标。此外,  $\gamma$ -分泌酶可以裂解细胞表面的 BCMA 形成可溶形式的 BCMA (sBCMA), sBCMA 由 BCMA 胞外结构域和一部分跨膜结构域组成, 它不仅降低了靶抗原的密度, 而且还会作为一种可溶性诱饵抵抗免疫细胞。因此 BCMA 靶向抗体联用  $\gamma$ -分泌酶抑制剂也是一种新的治疗模式。

吉满生物 H\_BCMA Reporter 293 Cell Line 是一种 Luciferase 报告基因细胞系。当 BAFF 或 APRIL 结合 BCMA 受体后, 激活下游信号通路, 从而激活荧光素酶 (Luciferase) 的表达, 使用阻断型的抗体可以阻断这一信号的传导。Luciferase 读值即代表信号通路的激活效果, 因此可用于 BCMA 相关药物的体外效果评价。

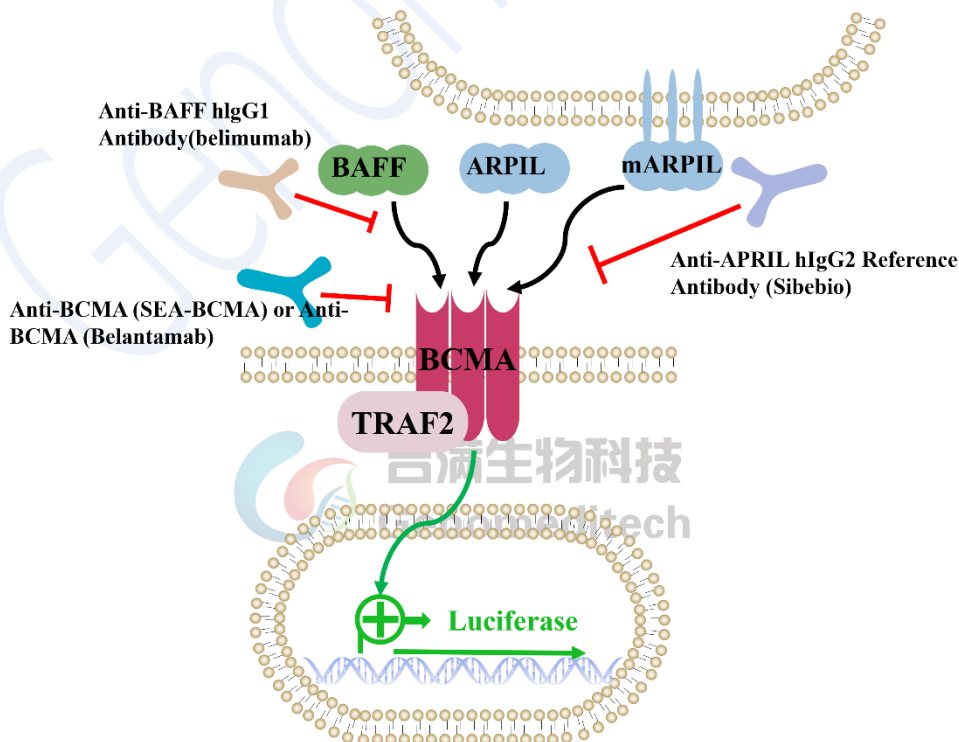


Fig 1. 信号通路图

## 四、 材料准备

### 1. 细胞培养、冻存、复苏试剂准备

|               |                                                              |
|---------------|--------------------------------------------------------------|
| 细胞复苏培养基:      | DMEM+10% FBS+1% P.S                                          |
| 细胞生长培养基:      | DMEM+10% FBS+1% P.S+4 µg/mL Blasticidin+0.75 µg/mL Puromycin |
| 细胞冻存液:        | 90% FBS+10% DMSO                                             |
| Assay Buffer: | DMEM+1% FBS+1% P.S                                           |

### 2. 试剂耗材准备

#### 试剂准备

| Reagent                                                       | Specification       | Manufacturer/Catalogue No. |
|---------------------------------------------------------------|---------------------|----------------------------|
| DMEM                                                          | 500 mL              | Gibco/C11995500BT          |
| Fetal Bovine Serum                                            | 500 mL              | ExCell/FSP500              |
| Pen/Strep                                                     | 500 mL              | Thermo/15140-122           |
| Blasticidin                                                   | 10 mg               | Genomeditech /GM-040404-1  |
| Puromycin                                                     | 25 mg               | Genomeditech/GM-040401-1   |
| 96 well round cell culture plate                              | Plate               | PakGent/CL-PT096           |
| 96-well White Opaque Plate                                    | Plate               | Thermo/236108              |
| 96-well U-bottom Plate                                        | Plate               | Saining/1014010            |
| Membrane Bound H <sub>2</sub> APRIL(Trimer) HEK-293 Cell Line | 1 管 ( 5E6 Cells/mL) | Genomeditech/GM-C40503     |
| Human BAFF Protein; His Tag                                   | /                   | Genomeditech/GM-87735RP    |
| Recombinant Human APRIL (N-Flag-His)                          | 50µg                | Novoprotein/CU89           |
| Anti-BCMA hIgG1 Antibody(Belantamab)                          | /                   | Genomeditech/GM-52396AB    |
| Anti-TNFSF13B(BAFF) hIgG1 Reference Antibody (Belibio)        | /                   | Genomeditech/GM-87985MAB   |
| Anti-APRIL hIgG2 Reference Antibody (Sibebio)                 | /                   | Genomeditech/GM-88014MAB   |
| Anti-BCMA hIgG1 Antibody(SEA-BCMA)                            | /                   | Genomeditech/GM-49486AB    |
| GMOne-Step 2.0 Luciferase Reporter Gene Assay Kit             | 1000T               | Genomeditech/GM-040513C    |

#### 重要仪器

| Equipment | Manufacturer/Catalogue No.         |
|-----------|------------------------------------|
| 细胞计数仪     | ThermoFisher Scientific/Countess 3 |
| 酶标仪       | Moleculardevices/SpectraMax L      |

## 五、细胞复苏、传代、冻存

### 1. 细胞复苏

注：为确保最高存活率，应在收到冻存细胞后立即解冻并复苏培养。如果在收到细胞后需要继续储存，将其置于液氮罐中，严禁储存在-70℃，因为在-70℃下储存会导致活性丧失。

- 37℃水浴锅预热复苏培养基，加入预热后的复苏培养基 5 mL 至 15 mL 离心管。
- 从液氮中取出冻存细胞并迅速放入 37℃恒温水浴锅，将细胞液面浸至水面以下轻轻摇动解冻，直到刚刚融化（通常 2-3 分钟）。
- 用 70%乙醇擦拭冻存管外部以降低污染的几率。在生物安全柜或超净台中将冻存管中的细胞悬液转移到步骤 a) 的离心管中，轻轻混匀， $176 \times g$ ，离心 5 min，使细胞沉淀，弃上清。
- 使用 1 mL 复苏培养基重悬，可取出部分使用台盼蓝染色计数活细胞，活细胞  $\geq 3 \times 10^6$  cells/mL。
- 通过补充复苏培养基的形式，调整活细胞密度到  $2-3 \times 10^5$  cells/mL，根据细胞悬液总体积，将细胞接种到合适的培养皿中。

### 3. 细胞冻存

- 使用  $176 \times g$ ，3 min 离心收集细胞。
- 使用预冷细胞冻存液（90% FBS + 10% DMSO）重悬细胞，细胞密度调整为  $5 \times 10^6$  cells/mL，每管 1 mL 分装到细胞冻存管中。
- 拧紧盖子，适当标记后，将冻存管置于梯度降温盒中，-80℃下保存至少 1 天，尽快转移至液氮中。

### 2. 细胞传代（以 10 cm 皿为例）

注：细胞复苏后的 1 至 2 代，使用复苏培养基，待细胞状态稳定后，再更换为含有抗生素的生长培养基。

- 细胞为上皮细胞，贴壁生长。培养箱中孵育 16-24 h 后，镜下观察细胞贴壁情况，当细胞密度达到 80%，需要进行细胞传代。推荐细胞传代比例为 1:3-1:4，2-3 天传代。注意保持密度不超过 80%，否则可能会因细胞受到挤压而导致活性减弱。
- 将皿或培养瓶中的培养液弃去，10 cm 皿加 2 mL PBS 润洗 1 次。
- 弃 PBS，加 1 mL 0.25% Trypsin-EDTA 消化液，37℃消化 30-60 s，显微镜下观察。
- 待细胞变圆，细胞间隙明显，部分细胞刚开始脱离瓶壁时，加 2 mL 左右生长培养基混匀终止消化，将细胞小心吹打下来， $176 \times g$  室温离心 3 min。
- 弃上清，细胞沉淀用生长培养基重悬，根据传代前细胞密度分盘（根据培养皿面积和细胞密度计算，传代后细胞密度为 30-40%）。

注意事项：

- 细胞刚复苏，会有一定比例的死细胞，属于正常现象，经调整会有明显好转，状态稳定后，传代后死细胞会变少，细胞生长速度趋于稳定。
- 注意保持密度不超过 80%，否则可能会因细胞受到挤压而导致活性减弱。
- FBS 需 56℃水浴 30 分钟，可热灭活补体和部分病毒，但不显著影响大多数生长因子和细胞因子活性。

## 六、 使用方法（示例）

### 1. 配体激活验证实验——BAFF

操作步骤可调整优化，对于本实验，推荐目的细胞 H\_BCMA Reporter 293 Cell Line 细胞量为  $1.5 \times 10^4$  cells/孔。使用 Human BAFF Protein; His Tag (19.0 KDa; 以下简称为 Human BAFF) 作为激活药物。起始浓度(Conc.01)为 10  $\mu\text{g/mL}$ ，3 倍梯度稀释，Conc.01-Conc.09 分别排布在 B2-B10，B11 为 0 浓度对照。周围为 100  $\mu\text{L}$  PBS，以防止边孔蒸发。

孔板排布如下：

|   |            | 1   | 2                      | 3                     | 4                     | 5            | 6            | 7           | 8           | 9          | 10         | 11  | 12  |
|---|------------|-----|------------------------|-----------------------|-----------------------|--------------|--------------|-------------|-------------|------------|------------|-----|-----|
| A |            | PBS | PBS                    | PBS                   | PBS                   | PBS          | PBS          | PBS         | PBS         | PBS        | PBS        | PBS | PBS |
| B | Human BAFF | PBS | 10.00 $\mu\text{g/mL}$ | 3.33 $\mu\text{g/mL}$ | 1.11 $\mu\text{g/mL}$ | 370.37 ng/mL | 123.46 ng/mL | 41.15 ng/mL | 13.72 ng/mL | 4.57 ng/mL | 1.52 ng/mL | 0   | PBS |
| C |            | PBS | PBS                    | PBS                   | PBS                   | PBS          | PBS          | PBS         | PBS         | PBS        | PBS        | PBS | PBS |
| D |            |     |                        |                       |                       |              |              |             |             |            |            |     |     |
| E |            |     |                        |                       |                       |              |              |             |             |            |            |     |     |
| F |            |     |                        |                       |                       |              |              |             |             |            |            |     |     |
| G |            |     |                        |                       |                       |              |              |             |             |            |            |     |     |
| H |            |     |                        |                       |                       |              |              |             |             |            |            |     |     |

#### 1) 加样步骤

- 在实验前 16-24 h，将细胞从培养瓶中取出，消化离心收集细胞沉淀，使用适量完全培养基重悬细胞，检测细胞活力并计数，再以完全培养基调整细胞浓度为  $1.5 \times 10^5$  cells/mL，以排枪加 100  $\mu\text{L}$  细胞/孔至中间孔，周围的孔加 100  $\mu\text{L}$  PBS。盖上板盖，于孵箱中孵育过夜使用。
- 使用 1 个无菌 96 孔 U 底板准备抗体稀释。
- 每个待测药物，使用一行（如 B2-B11）。
- 准备母液

| 药物名称       | 储液         | 母液 | 配置方法   |
|------------|------------|----|--------|
| Human BAFF | 1.56 mg/mL | /  | 直接使用储液 |

- 96 孔 U 底板中，加入 Assay Buffer，各孔体积见下表，如 B2 孔加入 180  $\mu\text{L}$  Assay Buffer，B3-B11 孔，加入 120  $\mu\text{L}$  Assay Buffer。

- f) 吸取不同体积的待测样品母液，加入到第一个梯度稀释孔中（如 B2 中加入 1.16  $\mu\text{L}$  Human BAFF），混匀。

| 母液吸取 |                               | 梯度稀释孔，依次从前孔吸取 60 $\mu\text{L}$ ，加入次孔 |                   |                   |                   |                   |                   |                   |                   |                   |                   | 对照孔               |    |
|------|-------------------------------|--------------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|----|
|      |                               | 1                                    | 2                 | 3                 | 4                 | 5                 | 6                 | 7                 | 8                 | 9                 | 10                | 11                | 12 |
| A    |                               |                                      |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |    |
| B    | 1.16 $\mu\text{L}$ Human BAFF | 加入                                   | 180 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ | 120 $\mu\text{L}$ |    |
| C    |                               |                                      |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |    |
| D    |                               |                                      |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |    |
| E    |                               |                                      |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |    |
| F    |                               |                                      |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |    |
| G    |                               |                                      |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |    |
| H    |                               |                                      |                   |                   |                   |                   |                   |                   |                   |                   |                   |                   |    |

- g) 从第一个梯度稀释孔 B2 中吸取 60  $\mu\text{L}$ ，加入到第二个梯度稀释孔 B3，充分混匀。
- h) 以此类推，直至第 9 个梯度稀释孔（B10）。
- i) 将步骤 a 准备好的孔板取出，吸弃上清。
- j) 加入步骤 h 准备好的梯度稀释液，每孔 100  $\mu\text{L}$ 。
- k) 盖上班盖，于 37°C CO<sub>2</sub> 培养箱中培养 6 h。
- l) 收样，转至 96 孔白底板上机，检测 Luciferase。

## 2) 报告基因检测

参考报告基因检测说明书。

| H_BCMA Reporter 293 Cell Line | 0 $\mu\text{g/mL}$ | 10.00 $\mu\text{g/mL}$ | 1.52 ng/mL |
|-------------------------------|--------------------|------------------------|------------|
|                               | 81023              | 24266103               | 105880     |

## 3) 验证结果

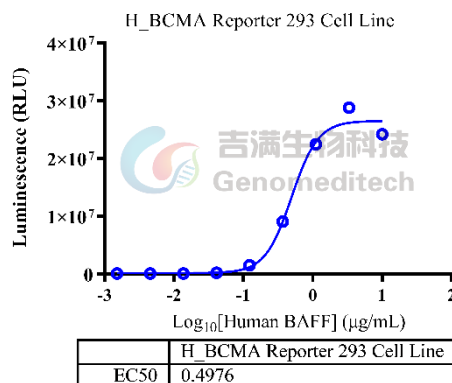


Fig 2. Response to Human BAFF Protein; His Tag. The H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) at a density of  $1.5 \times 10^4$  cells/well (96-well format) was stimulated with serial dilutions of Human BAFF Protein; His Tag (Cat. GM-87735RP) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech).

## 2. BAFF 激活，抗体 block 实验

操作步骤可调整优化，对于本实验，推荐 H\_BCMA Reporter 293 Cell Line 细胞量为  $1.5 \times 10^4$  Cells/孔。使用 Human BAFF Protein; His Tag 作为激活剂（以下简称为 BAFF），使用 Anti-BCMA hIgG1 Antibody (Belantamab) (以下简称为 Belantamab；约 150 kDa) 作为阳性药物，Conc.01 终浓度为  $30 \mu\text{g/mL}$ ，3 倍梯度稀释，Conc.01-Conc.09 分别排布在 B2-B10，B11 为 0 浓度对照。周围为  $100 \mu\text{L}$  PBS，以防止边孔蒸发。

孔板布局：

|   | 1          | 2   | 3           | 4              | 5             | 6             | 7               | 8               | 9              | 10             | 11            | 12         |     |
|---|------------|-----|-------------|----------------|---------------|---------------|-----------------|-----------------|----------------|----------------|---------------|------------|-----|
| A |            | PBS | PBS         | PBS            | PBS           | PBS           | PBS             | PBS             | PBS            | PBS            | PBS           | PBS        |     |
| B | Belantamab | PBS | 30<br>µg/mL | 10.00<br>µg/mL | 3.33<br>µg/mL | 1.11<br>µg/mL | 370.37<br>ng/mL | 123.46<br>ng/mL | 41.15<br>ng/mL | 13.72<br>ng/mL | 4.57<br>ng/mL | 0<br>µg/mL | PBS |
| C |            | PBS | PBS         | PBS            | PBS           | PBS           | PBS             | PBS             | PBS            | PBS            | PBS           | PBS        |     |
| D |            |     |             |                |               |               |                 |                 |                |                |               |            |     |
| E |            |     |             |                |               |               |                 |                 |                |                |               |            |     |
| F |            |     |             |                |               |               |                 |                 |                |                |               |            |     |
| G |            |     |             |                |               |               |                 |                 |                |                |               |            |     |
| H |            |     |             |                |               |               |                 |                 |                |                |               |            |     |

### 1) 加样步骤

- 在实验前 16-24 h，将细胞从培养瓶中取出，消化离心收集细胞沉淀，使用适量完全培养基重悬细胞，检测细胞活力并计数，再以完全培养基调整细胞浓度为  $1.5 \times 10^5$  cells/mL。以排枪加  $100 \mu\text{L}$  细胞/孔至中间孔。周围的孔加  $100 \mu\text{L}$  PBS。盖上板盖，于孵箱中孵育过夜使用。
- 使用 1 个无菌 96 孔 U 底板准备抗体稀释。
- 每个待测抗体，使用一行（如 B2-B10）。
- 准备母液

| 抗体名称       | 储液         | 母液 | 配置方法   |
|------------|------------|----|--------|
| Belantamab | 4.16 mg/mL | /  | 直接使用储液 |
| BAFF       | 1.56 mg/mL | /  | 直接使用储液 |

- e) 96 孔 U 底板中，加入 Assay Buffer，各孔体积见下表，如 B2 孔加入 90  $\mu\text{L}$  Assay Buffer；B3-B11 孔分别加入 60  $\mu\text{L}$  Assay Buffer。
- f) 吸取不同体积的待测样品母液，加入到第一个梯度稀释孔中（如 B2 加入 1.32  $\mu\text{L}$  Belantamab），混匀。

| 母液吸取 |                    | 梯度稀释孔，依次从前孔吸取 30 μL，加入次孔 |       |       |       |       |       |       |       |       |       | 对照孔   |    |
|------|--------------------|--------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|----|
|      |                    | 1                        | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12 |
| A    |                    |                          |       |       |       |       |       |       |       |       |       |       |    |
| B    | 1.32 μL Belantamab | 加入                       | 90 μL | 60 μL | 60 μL | 60 μL | 60 μL | 60 μL | 60 μL | 60 μL | 60 μL | 60 μL |    |
| C    |                    |                          |       |       |       |       |       |       |       |       |       |       |    |
| D    |                    |                          |       |       |       |       |       |       |       |       |       |       |    |
| E    |                    |                          |       |       |       |       |       |       |       |       |       |       |    |
| F    |                    |                          |       |       |       |       |       |       |       |       |       |       |    |
| G    |                    |                          |       |       |       |       |       |       |       |       |       |       |    |
| H    |                    |                          |       |       |       |       |       |       |       |       |       |       |    |

- g) 从第一个梯度稀释孔 B2 中吸取 30  $\mu\text{L}$ ，加入到第二个梯度稀释孔 B3，充分混匀。
- h) 以此类推，直至第 9 个梯度稀释孔（B10）。
- i) 将步骤 a 孵育过夜的孔板取出，吸弃上清。加入步骤 h 梯度稀释的抗体，50  $\mu\text{L}$  每孔，与细胞孵育 1h。
- j) 配置  $2 \times$  激活剂，1000 ng/mL BAFF（1.27  $\mu\text{L}$  1.56 mg/mL BAFF 母液加入到 1982  $\mu\text{L}$  Assay Buffer 中，混匀后使用）。
- k) 1 h 后，将步骤 i 的细胞孔板取出，然后加入步骤 j 配置好的 BAFF 溶液，每孔加入 50  $\mu\text{L}$ 。
- l) 盖上板盖，于 37  $^{\circ}\text{C}$   $\text{CO}_2$  培养箱中培养 6 h。
- m) 收样，转至 96 孔白底板上机，检测 Luciferase。

## 2) 报告基因检测

参考报告基因检测说明书。

| H_BCMA Reporter<br>293 Cell Line | 500 ng/mL BAFF + 0 $\mu\text{g/mL}$<br>Belantamab | 500 ng/mL BAFF + 30<br>$\mu\text{g/mL}$ Belantamab | 500 ng/mL BAFF +<br>4.57 ng/mL Belantamab |
|----------------------------------|---------------------------------------------------|----------------------------------------------------|-------------------------------------------|
|                                  | 24516340                                          | 102373                                             | 21864159                                  |

### 3) 验证结果

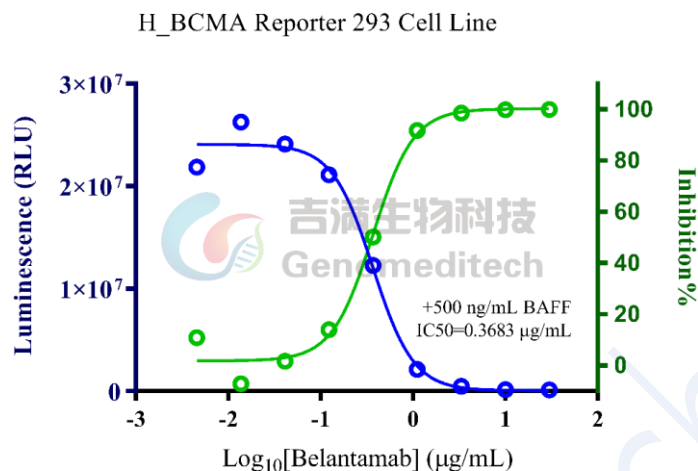


Fig 3. Inhibition of BAFF Protein-induced reporter activity by Belantamab. H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) was seeded at a density of  $1.5 \times 10^4$  cells per well in a 96-well plate and incubated overnight. The next day, Serial dilutions of the Anti-BCMA hIgG1 Antibody (Belantamab)(Cat. GM-52396AB) was incubated with  $1.5 \times 10^4$  cells/well of the H\_BCMA Reporter 293 Cell Line in a 96-well plate for 1 hour in assay buffer. Subsequently, the Human BAFF Protein (Cat. GM-87735RP) at a density of 50 ng/well was added, and the coculture proceeded for an additional 6 hours. Firefly luciferase activity was then measured using the Luciferase Reporter Assay Kit (Genomeditech). (left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

### 3. 共培养激活后，Anti-APRIL 抗体 block 实验

操作步骤可调整优化，本实验使用  $1.5 \times 10^4$  cells/Well 的 H\_BCMA Reporter 293 Cell Line 和  $1 \times 10^4$  cells/Well 的 Membrane Bound H\_APRIL(Trimer) HEK-293 Cell Line 进行实验。本次实验使用 Anti-APRIL hIgG2 Reference Antibody (Sibebio)（以下简称 Sibeprenlimab; 约 150 kDa）作为阳性药物，Conc.01 终浓度为 10 μg/mL，4 倍梯度稀释，Conc.01-Conc.09 分别排布在 B2-B10，B11 为 0 浓度对照。周围孔加入 100 μL PBS，以防止边孔蒸发。

孔板排布如下：

|   | 1             | 2   | 3              | 4             | 5               | 6               | 7              | 8             | 9             | 10              | 11              | 12         |
|---|---------------|-----|----------------|---------------|-----------------|-----------------|----------------|---------------|---------------|-----------------|-----------------|------------|
| A |               | PBS | PBS            | PBS           | PBS             | PBS             | PBS            | PBS           | PBS           | PBS             | PBS             | PBS        |
| B | Sibeprenlimab | PBS | 10.00<br>μg/mL | 2.50<br>μg/mL | 625.00<br>ng/mL | 156.25<br>ng/mL | 39.06<br>ng/mL | 9.77<br>ng/mL | 2.44<br>ng/mL | 610.35<br>pg/mL | 152.59<br>pg/mL | 0<br>pg/mL |
| C |               |     | PBS            | PBS           | PBS             | PBS             | PBS            | PBS           | PBS           | PBS             | PBS             | PBS        |
| D |               |     |                |               |                 |                 |                |               |               |                 |                 |            |
| E |               |     |                |               |                 |                 |                |               |               |                 |                 |            |
| F |               |     |                |               |                 |                 |                |               |               |                 |                 |            |

|   |  |  |  |  |  |  |  |  |  |  |  |  |
|---|--|--|--|--|--|--|--|--|--|--|--|--|
| G |  |  |  |  |  |  |  |  |  |  |  |  |
| H |  |  |  |  |  |  |  |  |  |  |  |  |

## 1) 加样步骤

a) 实验前 16-24 h, 消化离心收集 H\_BCMA Reporter 293 Cell Line 细胞, 用适量完全培养基重悬细胞, 检测细胞活力并计数, 再以完全培养基调整细胞密度到  $1.5 \times 10^5$  cells/mL, 以排枪加 100  $\mu$ L 细胞/孔至中间孔, 周围的孔加 100  $\mu$ L PBS, 盖上板盖, 于孵箱中孵育过夜。

b) 使用 1 个无菌 96 孔 U 底板准备抗体稀释。

c) 对于待测样品, 使用一行 (如 B 行)。

d) 母液配置

| 药物名称          | 储液          | 母液          | 配置方法                                   |
|---------------|-------------|-------------|----------------------------------------|
| Sibeprenlimab | 17.46 mg/mL | 1.746 mg/mL | 取 2 $\mu$ L 储液+18 $\mu$ L Assay Buffer |

e) 96 孔 U 底板中, 加入 Assay Buffer, 各孔体积见下表, 如 B2 孔加入 80  $\mu$ L Assay Buffer, B3-B11 孔, 加入 60  $\mu$ L Assay Buffer。

f) 吸取不同体积的待测样品母液, 加入到第一个梯度稀释孔中 (如 B2 中加入 0.93  $\mu$ L Sibeprenlimab), 混匀。

| 母液吸取 |                               | 梯度稀释孔, 依次从前孔吸取 20 $\mu$ L, 加入次孔 |            |            |            |            |            |            |            |            |            | 对照孔        |    |
|------|-------------------------------|---------------------------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|----|
|      |                               | 1                               | 2          | 3          | 4          | 5          | 6          | 7          | 8          | 9          | 10         | 11         | 12 |
| A    |                               |                                 |            |            |            |            |            |            |            |            |            |            |    |
| B    | 0.93 $\mu$ L<br>Sibeprenlimab | 加入                              | 80 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L | 60 $\mu$ L |    |
| C    |                               |                                 |            |            |            |            |            |            |            |            |            |            |    |
| D    |                               |                                 |            |            |            |            |            |            |            |            |            |            |    |
| E    |                               |                                 |            |            |            |            |            |            |            |            |            |            |    |
| F    |                               |                                 |            |            |            |            |            |            |            |            |            |            |    |
| G    |                               |                                 |            |            |            |            |            |            |            |            |            |            |    |
| H    |                               |                                 |            |            |            |            |            |            |            |            |            |            |    |

g) 从第一个梯度稀释孔 (如 B2 孔) 中吸取 20  $\mu$ L 液体, 加入到第二个稀释孔中 (如 B3), 充分混匀。

h) 以此类推, 直至第 9 个梯度稀释孔 (B10)。

i) 实验前 1-2 h, 消化离心收集 Membrane Bound H\_APRIL(Trimer) HEK-293 Cell Line 溶液, 使用 Assay Buffer 调整细胞密度到  $1 \times 10^5$  cells/mL。

- j) 取出步骤 a 准备好的 H\_BCMA Reporter 293 Cell Line 细胞，吸弃上清。
- k) 加入步骤 h 准备好稀释抗体，每孔 50  $\mu\text{L}$ ；然后再加入步骤 i 准备好的 Membrane Bound H\_APRIL(Trimer) HEK-293 Cell Line 溶液，每孔 50  $\mu\text{L}$ 。
- l) 盖上板盖，于 37  $^{\circ}\text{C}$  CO<sub>2</sub> 培养箱中培养 6 h。
- m) 收样，转至 96 孔白底板上机，检测 Luciferase。

## 2) 报告基因检测

参考报告基因检测说明书。

|                                                                                        |                    |                        |                         |
|----------------------------------------------------------------------------------------|--------------------|------------------------|-------------------------|
| H_BCMA Reporter 293 Cell Line +<br>Membrane Bound H_APRIL(Trimer)<br>HEK-293 Cell Line | 0 $\mu\text{g/mL}$ | 10.00 $\mu\text{g/mL}$ | 152.59 $\mu\text{g/mL}$ |
|                                                                                        | 32206825           | 141504                 | 28166863                |

## 3) 验证结果

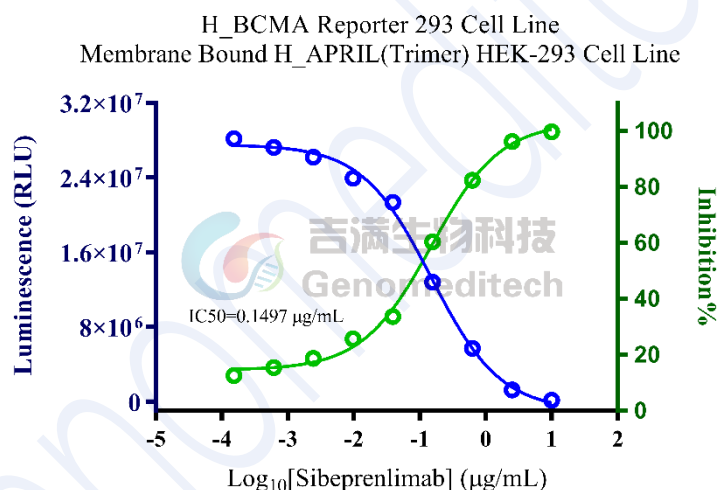


Fig 4. Inhibition of Membrane Bound H\_APRIL(Trimer) 293-induced reporter activity by Sibeprenlimab. The H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, Serial dilutions of the Anti-APRIL hIgG2 Reference Antibody (Sibebio) (Cat. GM-88014MAB) and 1E4 cells/well of the Membrane Bound H\_APRIL(Trimer) HEK-293 Cell Line (Cat. GM-C40503) were added to 1.5E4 cells/well of H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). (left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis. Data are shown by drug mass concentration.

## 附录 1 BAFF Protein 激活, Anti-BAFF 抗体 block

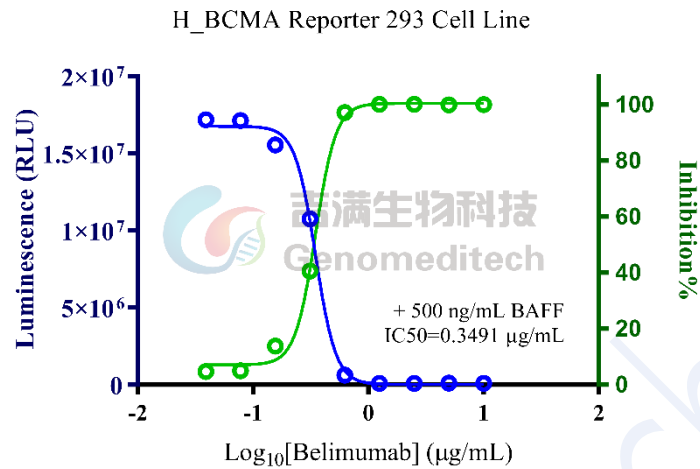


Fig 5. Inhibition of BAFF Protein-induced reporter activity by Belimumab. H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, Serial dilutions of Anti-TNFSF13B(BAFF) hIgG1 Reference Antibody (Belibio) (Cat. GM-87985MAB) was incubated with 50 ng/mL of Human BAFF Protein(Cat. GM-87735RP) for 1 hour in assay buffer. After pre-incubation, add to the pre-seeded cells, and incubate for 6 hours. Firefly luciferase activity was then measured using the Luciferase Reporter Assay Kit (Genomeditech). (left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis. Data are shown by drug mass concentration.

## 附录 2 APRIL Protein 激活结果

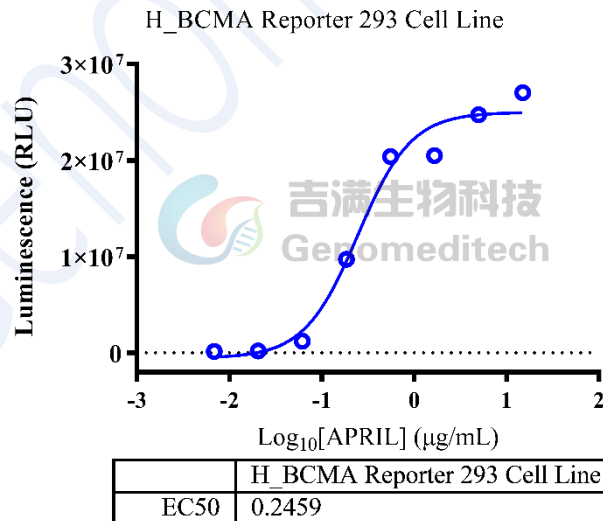


Fig 6. Response to Recombinant Human APRIL (N-Flag-His). The H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) at a density of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Recombinant Human APRIL (N-Flag-His)(Novoprotein/CU89) in assay buffer (DMEM+1% FBS+1% P.S) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech). The maximum induction fold was approximately [180.2]. Data are shown by drug mass concentration.

### 附录3 BAFF Protein 激活后, Anti-BCMA 抗体 block

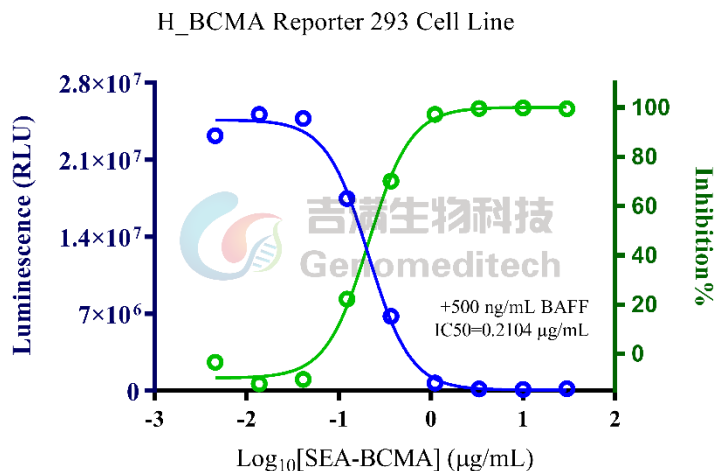


Fig 7. Inhibition of BAFF Protein-induced reporter activity by SEA-BCMA. H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, Serial dilutions of the Anti-BCMA hIgG1 Antibody(SEA-BCMA)(Cat. GM-49486AB) was incubated with 1.5E4 cells/well of the H\_BCMA Reporter 293 Cell Line in a 96-well plate for 1 hour in assay buffer. Subsequently, the Human BAFF Protein (Cat. GM-87735RP) at a density of 50 ng/well was added, and the coculture proceeded for an additional 6 hours. Firefly luciferase activity was then measured using the Luciferase Reporter Assay Kit (Genomeditech). (left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

### 附录4 共培养激活结果

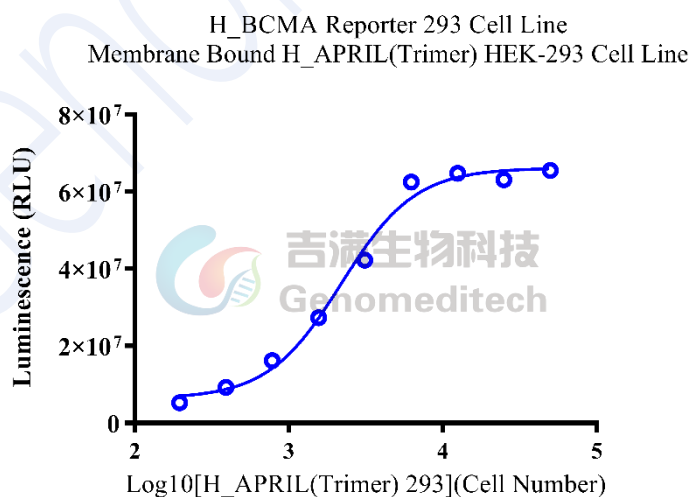


Fig 8. Response to Membrane Bound H\_APRIL(Trimer) HEK-293 Cell Line. H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) at a concentration of 1.5E4 cells/well (96-well format) was stimulated with serial dilutions of Membrane Bound H\_APRIL(Trimer) HEK-293 Cell Line (Cat. GM-C40503) for 6 hours. The firefly luciferase activity was measured using the Luciferase Reporter Assay Kit (Genomeditech).

## 附录 5 共培养激活后，使用 Anti-BCMA 抗体 block

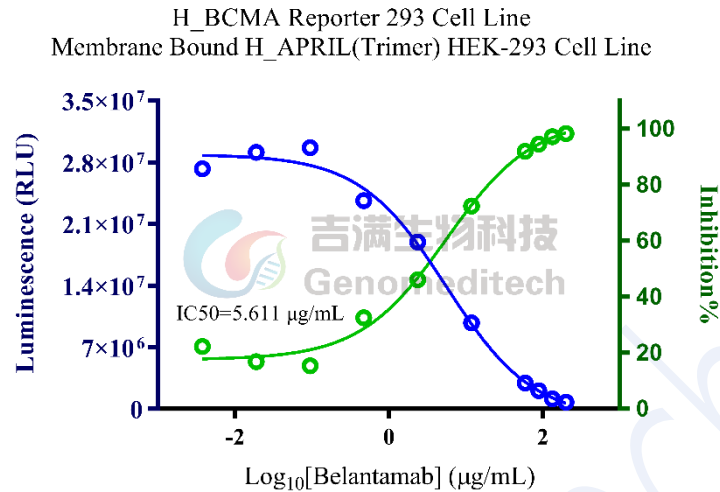


Fig 9. Belantamab inhibits membrane-bound H\_APRIL(Trimer)-induced reporter activity in H\_BCMA Reporter 293 cells. The H\_BCMA Reporter 293 (Cat. GM-C44705) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, H\_BCMA Reporter 293 Cells were incubated with serial dilutions of Anti-BCMA hIgG1 Antibody(Belantamab) (Cat. GM-52396AB) (S1-S4: 1.5-fold from 200 μg/mL; S4-S10: 5-fold from 59.26 μg/mL) for 1 hours, then co-cultured with Membrane Bound H\_APRIL(Trimer) HEK-293(Cat. GM-C40503) for 6 hours. Firefly luciferase activity was then measured using the Luciferase Reporter Assay Kit (Genomeditech) (left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

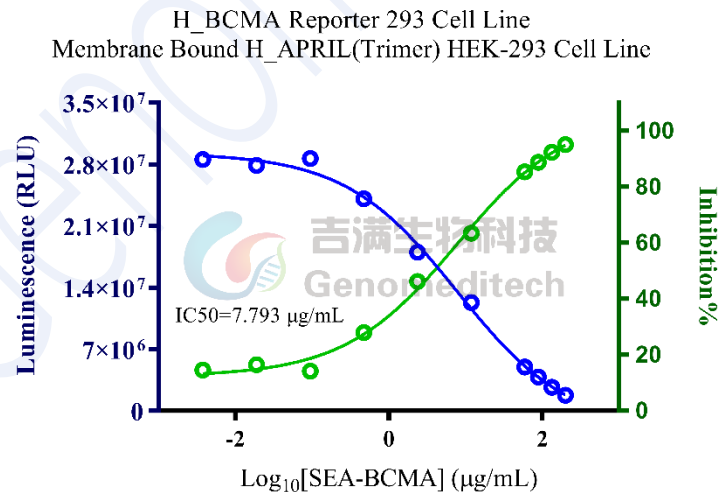


Fig 10. SEA-BCMA inhibits membrane-bound H\_APRIL(Trimer)-induced reporter activity in H\_BCMA Reporter 293 cells. The H\_BCMA Reporter 293 (Cat. GM-C44705) was seeded at a density of 1.5E4 cells per well in a 96-well plate and incubated overnight. The next day, H\_BCMA Reporter 293 Cells were incubated with serial dilutions of Anti-BCMA hIgG1 Antibody(SEA-BCMA) (Cat. GM-49486AB) (S1-S4: 1.5-fold from 200 μg/mL; S4-S10: 5-fold from 59.26 μg/mL) for 1 hours, then co-cultured with Membrane Bound H\_APRIL(Trimer) HEK-293(Cat. GM-C40503) for 6 hours. Firefly luciferase activity was then measured using the Luciferase Reporter

Assay Kit (Genomeditech) (left Y-axis, relative luminescence units), with inhibition percentages shown on the right Y-axis.

## 附录 6 流式验证结果

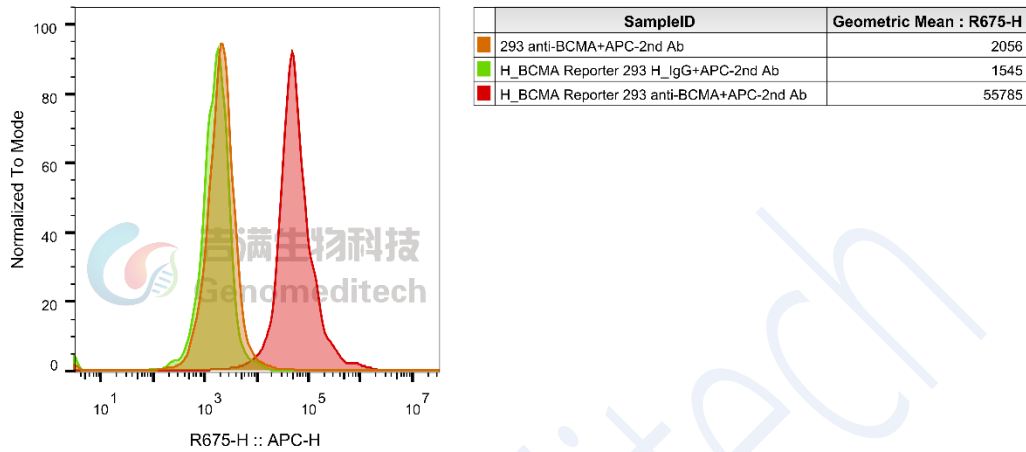


Fig 11. H\_BCMA Reporter 293 Cell Line (Cat. GM-C44705) was determined by flow cytometry using Anti-BCMA hIgG1 Antibody(Belantamab) (Cat. GM-52396AB).

## 相关产品

| CD40: CD40L                                  |                                           |
|----------------------------------------------|-------------------------------------------|
| H_CD40(TNFRSF5) Reporter 293 Cell Line       | H_CD40(TNFRSF5) Reporter Jurkat Cell Line |
| Cynomolgus_CD40 CHO-K1 Cell Line             | Cynomolgus_CD40L CHO-K1 Cell Line         |
| H_CD40(TNFRSF5) CHO-K1 Cell Line             | H_CD40(TNFRSF5) HEK-293 Cell Line         |
| H_CD40L CHO-K1 Cell Line                     | H_CD40L HEK-293 Cell Line                 |
| Anti-H_CD40 hIgG1 Antibody(APX005M)          | Anti-H_CD40 hIgG1 Antibody(ravagalimab)   |
| Anti-H_CD40L hIgG1 Antibody(dapirolizumab)   | Anti-H_CD40L hIgG1 Antibody(frexalimab)   |
| IFN-α                                        |                                           |
| IFNα Reporter HEK-293 Cell Line              | IFNα Reporter MDCK Cell Line              |
| IFNα Reporter THP1 Cell Line                 |                                           |
| BCMA:BCMA:TACI                               |                                           |
| H_BCMA Reporter 293 Cell Line                | H_TACI Reporter Cell Line                 |
| Cynomolgus_BCMA CHO-K1 Cell Line             | H_BCMA CHO-K1 Cell Line                   |
| H_BCMA HEK-293 Cell Line                     |                                           |
| Anti-BCMA hIgG1 Antibody(Belantamab)         | Anti-BCMA hIgG1 Antibody(SEA-BCMA)        |
| Anti-BCMA hIgG4 Antibody(BCMB69)             |                                           |
| Biotinylated Human BAFF Protein; His-Avi Tag | Cynomolgus BAFF Protein; His Tag          |
| Human BAFF Protein; His Tag                  | Mouse BAFF Protein; His Tag               |
| BDCA2(CLEC4C)                                |                                           |
| H_BDCA2 Reporter Jurkat Cell Line            | Cynomolgus_BDCA2 CHO-K1 Cell Line         |

|                                                      |                                             |
|------------------------------------------------------|---------------------------------------------|
| Cynomolgus_BDCA2 Jurkat Cell Line                    | H_BDCA2 CHO-K1 Cell Line                    |
| H_BDCA2 HEK-293 Cell Line                            | H_BDCA2 Jurkat Cell Line                    |
| Anti-H_BDCA2 hIgG1 Antibody(Litifilimab)             |                                             |
| Cynomolgus BDCA2 Protein; His Tag                    | Human BDCA2 Protein; His Tag                |
| CD3                                                  |                                             |
| Jurkat CD3-BsAb Reporter Cell Line                   | Cynomolgus_CD3 HEK-293 Cell Line            |
| Cynomolgus_CD3E(Membrane Bound ECD) CHO-K1 Cell Line | H_CD3 CHO-K1 Cell Line                      |
| H_CD3 HEK-293 Cell Line                              | H_CD3E(Membrane Bound ECD) CHO-K1 Cell Line |
| Mouse_CD3 HEK-293 Cell Line                          |                                             |
| Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]  | Anti-CD3 hIgG1 Antibody(CH2527)             |

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