一、 产品信息及背景

产品编号	产品名称	规格	数量
GM-C42136	H_PD1 SHP2 Reporter Jurkat Cell Line	5E6 Cells/mL	1 管

程序性死亡受体 1（PD-1，亦称 CD279）是一种表达于活化 T 细胞、B 细胞及部分髓系细胞表面的免疫检查点受体，属于 Ig 超家族，其胞外区与配体 PD-L1/PD-L2 结合后，通过胞内含免疫受体酪氨酸抑制基序（ITIM）与免疫受体酪氨酸开关基序（ITSM）的酪氨酸位点被磷酸化，进而招募含有 Src 同源 2 结构域（SH2）的蛋白酪氨酸磷酸酶，尤其是 SHP-2（PTPN11）及在特定情境下的 SHP-1（PTPN6）。SHP-2 主要在造血细胞中高表达，作为负向调节因子通过去磷酸化 TCR、BCR 及其下游信号分子，抑制 PI3K-AKT、RAS-MAPK 等通路，从而降低细胞活化与效应功能。在肿瘤微环境和慢性感染中，PD-1 与其配体持续作用可通过招募 SHP-1/2 抑制 T 细胞代谢与细胞毒性，导致“耗竭”表型，这一轴线构成免疫逃逸的重要机制。

吉满生物的 H_PD1 SHP2 Reporter Jurkat Cell Line 报告基因细胞系采用荧光素酶片段互补（EFC）技术进行检测。在受体被配体结合或抗体交联激活后，酪氨酸残基发生磷酸化，从而特异性招募含 SH2 结构域的磷酸酶 Src homology region 2 domain-containing phosphatase-2（SHP-2）。随后被招募的 SHP-2 对多种关键信号分子进行去磷酸化，抑制 T 细胞受体（T cell receptor, TCR）等活化信号通路的传导，最终导致细胞活化受抑。当加入荧光素酶底物后，催化底物反应，从而产生可检测的发光信号。当加入阻断抗体后可以抑制信号。因此可用于相关药物的体外效果评价。

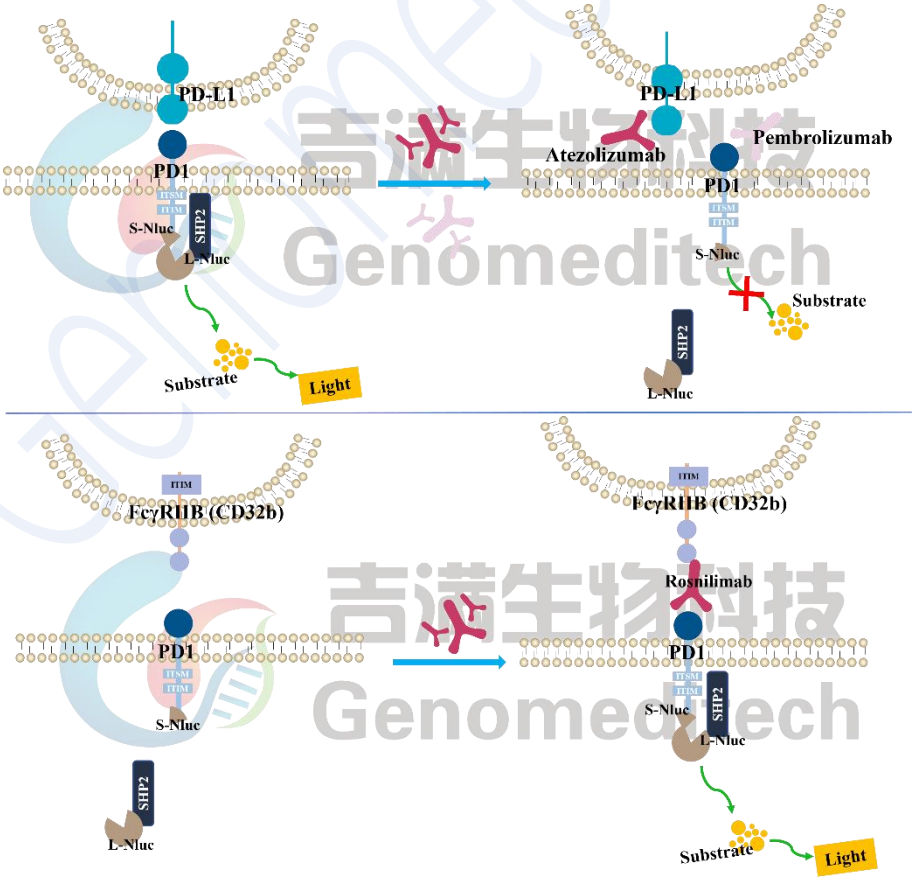
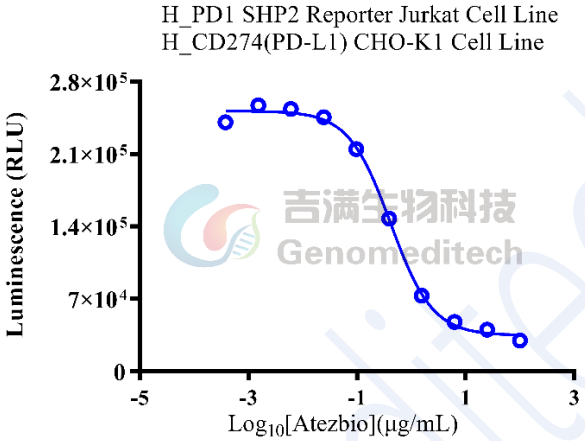


Fig 1 Signal pathway diagram

二、 包装、运输及储存

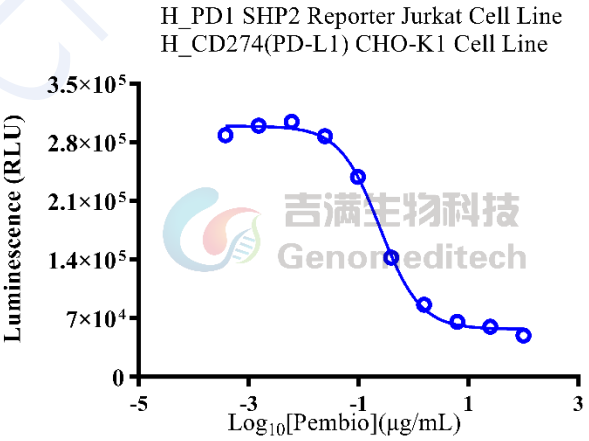
1. 细胞系产品干冰运输，–196°C 以下（冰箱或液氮的气相）长期储存。
2. 接触产品请带手套。请收到产品立即确认产品是否为冻存状态，–196°C 以下（冰箱或液氮的气相）长期储存。
3. 本产品相关实验，应在二级生物安全实验室或生物安全柜中进行。

三、 验证结果



	H_PD1 SHP2 Reporter Jurkat
IC50	0.4164

Fig 2. Response to Anti-H_PDL1 hIgG1 Reference Antibody(Atezbio). Serial dilutions of the Anti-H_PDL1 hIgG1 Reference Antibody(Atezbio) (Cat. GM-86854MAB) was incubated with 1.5E4 cells/well of the H_CD274(PD-L1) CHO-K1 Cell Line (Cat. GM-C01115) in a 96-well plate for 1 hour. Subsequently, H_PD1 SHP2 Reporter Jurkat Cell Line (Cat. GM-C42136) with a concentration of 1E5 cells/well was added, and the co-culture proceeded for an additional 6 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Luciferase activity was measured using the Luciferase Assay System. The results indicated a maximum fold of approximately [7.8]. Data are shown by drug mass concentration.

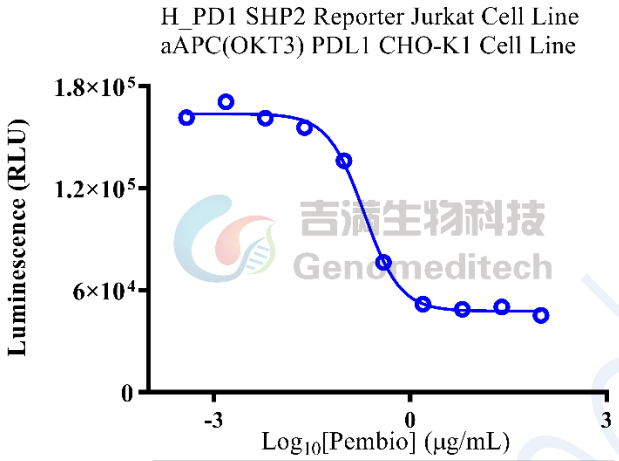


	H_PD1 SHP2 Reporter Jurkat
IC50	0.2456

Fig 3. Response to Anti-PD1 hIgG4 Reference Antibody (Pembio). H_CD274(PD-L1) CHO-K1 Cell Line (Cat. GM-C01115) was seeded at a density of 1.5E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-PD1 hIgG4 Reference Antibody (Pembio) (Cat. GM-87802MAB were incubated with

1E5 cells/well of the H_PD1 SHP2 Reporter Jurkat Cell Line (Cat. GM-C42136) in a 96-well plate for 1 hour, and then added to the pre-seeded cells. The mixture was incubated for an additional 6 hours.

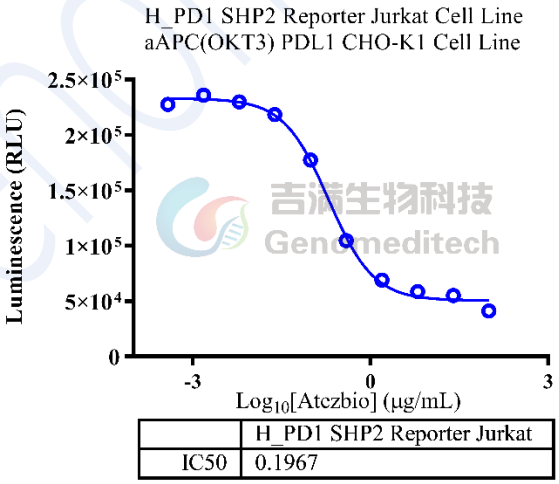
Luciferase activity was measured using the Luciferase Assay System. The results indicated a maximum fold of approximately [5.4]. Data are shown by drug mass concentration.



	H_PD1 SHP2 Reporter Jurkat
IC50	0.1965

Fig 4. Response to Anti-PD1 hIgG4 Reference Antibody (Pembio). aAPC(OKT3) PDL1 CHO-K1 Cell Line (Cat. GM-C05269) was seeded at a density of 1.5E4 cells/well in a 96-well plate and incubated overnight. The next day, serial dilutions of the Anti-PD1 hIgG4 Reference Antibody (Pembio) (Cat. GM-87802MAB were incubated with 1E5 cells/well of the H_PD1 SHP2 Reporter Jurkat Cell Line (Cat. GM-C42136) in a 96-well plate for 1 hour, and then added to the pre-seeded cells. The mixture was incubated for an additional 6 hours.

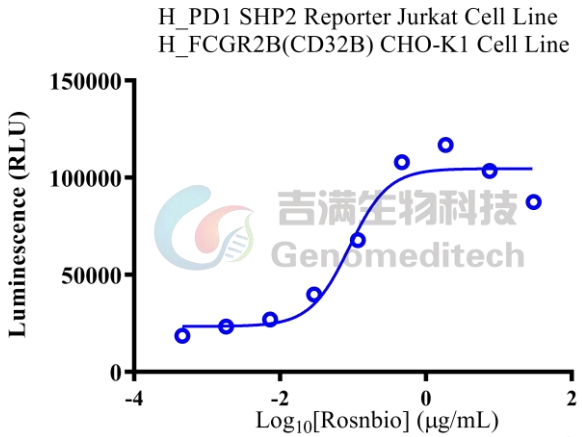
Luciferase activity was measured using the Luciferase Assay System. The results indicated a maximum fold of approximately [3.1]. Data are shown by drug mass concentration.



	H_PD1 SHP2 Reporter Jurkat
IC50	0.1967

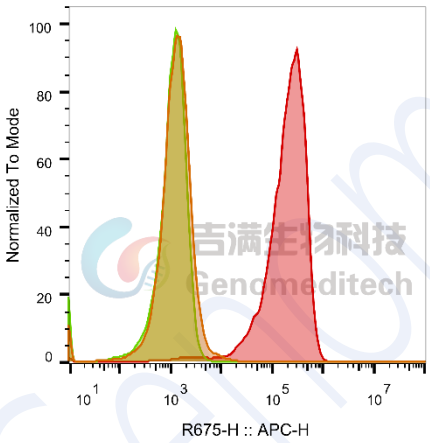
Fig 5. Response to Anti-H_PDL1 hIgG1 Reference Antibody(Atezbio). Serial dilutions of the Anti-H_PDL1 hIgG1 Reference Antibody(Atezbio) (Cat. GM-86854MAB) was incubated with 1.5E4 cells/well of the aAPC(OKT3) PDL1 CHO-K1 Cell Line (Cat. GM-C05269) in a 96-well plate for 1 hour. Subsequently, H_PD1 SHP2 Reporter Jurkat Cell Line (Cat. GM-C42136) with a concentration of 1E5 cells/well was added, and the co-culture proceeded for an additional 6 hours in assay buffer (RPMI 1640 + 1% FBS + 1% P.S).

Luciferase activity was measured using the Luciferase Assay System. The results indicated a maximum fold of approximately [4.6]. Data are shown by drug mass concentration.



	H_PD1 SHP2 Reporter Jurkat
EC50	0.08906

Fig 6. Response to Anti-PD1 hIgG1 Reference Antibody(Rosnbio). Serial dilutions of the Anti-PD1 hIgG1 Reference Antibody(Rosnbio) (Cat. GM-87930MAB)and 1E5 cells/well of the H_PD1 SHP2 Reporter Jurkat Cell Line (Cat. GM-C42136) were added to 1.5E4 cells/well of H_FCGR2B(CD32B) CHO-K1 Cell Line (Cat. GM-C16925) for 6 hours. Luciferase activity was measured using the Luciferase Assay System. The maximum induction fold was approximately [8.4]. Data are shown by drug mass concentration.



SampleID	Geometric Mean : R675-H
Jurkat Anti-PD1+APC 2nd Ab	1202
H_PD1 SHP2 Jurkat Anti-H_IgG+APC 2nd Ab	986
H_PD1 SHP2 Jurkat Anti-PD1+APC 2nd Ab	172885

Fig 7. H_PD1 SHP2 Reporter Jurkat Cell Line Cell Line (Cat. GM-C42136) Was determined by flow cytometry using Anti-PD1 hIgG1 Reference Antibody(Rosnbio) (Cat. GM-87930MAB).

四、 培养条件及试剂材料

细胞复苏培养基：	RPMI 1640+10% FBS+1% P.S
细胞生长培养基：	RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+0.75 μg/mL Puromycin
Assay Buffer：	RPMI 1640+1% FBS +1% P.S

Reagent	Specification	Manufacturer/Catalogue No.
Puromycin	25 mg	Genomeditech/GM-040401-1
Blasticidin	10 mg	Genomeditech /GM-040404-1
Pen/Strep	100 mL	Thermo/15140-122
Fetal Bovine Serum	500 mL	ExCell/FSP500
RPMI 1640	500 mL	Gibco/C11875500BT

96-well U-bottom Plate	96-well	Saining/1014010
96-well White Opaque Plate	96-well	Thermo/236108
96 well round cell culture plate	96-well	PakGent/CL-PT096
aAPC(OKT3) PDL1 CHO-K1 Cell Line	/	Genomeditech/GM-C05269
H_CD274(PD-L1) CHO-K1 Cell Line	/	Genomeditech/GM-C01115
Anti-PD1 hIgG4 Reference Antibody (Pembio)	/	Genomeditech/GM-87802MAB
Anti-H_PDL1 hIgG1 Reference Antibody (Atezbio)	/	Genomeditech/GM-86854MAB
Anti-PD1 hIgG1 Reference Antibody(Rosnbio)	/	Genomeditech/GM-87930MAB

五、 细胞复苏、传代、冻存

细胞复苏

a) 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。

b) 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）。

c) 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 × g，离心 5 min，使细胞沉淀，弃上清。

d) 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，细胞≥ 3×10^6 cells/mL。

e) 通过补加复苏培养基的形式，调整活细胞密度到 $4\text{-}6 \times 10^5$ cells/mL，根据细胞悬液总体积，将细胞悬液接种至 1-2 个 T25 中（3-5 mL 悬液），竖瓶培养。

细胞冻存

a) 使用 176 × g，3 min 离心收集细胞。

b) 使用预冷细胞冻存液（90% FBS + 10% DMSO）重悬细胞，细胞密度调整为 5×10^6 cells/mL，每管 1 mL 分装到细胞冻存管中。

c) 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。

细胞传代

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。

a) 此细胞为淋巴细胞状，悬浮生长。

b) 首次复苏后，约 48-72 h 可进行第一次传代，此次传代后细胞培养基可调整为添加抗生素的生长培养基。若 48 h 未传代，建议适当补加复苏培养基，瓶体改为横向放置。

c) 当细胞密度达到 $1.5\text{-}2 \times 10^6$ cells/mL，1 传 3，隔 2-3 天继续传代，不要让其密度超 2×10^6 cells/mL，推荐使用 T25 瓶进行传代培养。

d) 该细胞为悬浮细胞，传代时推荐使用【半换液法】对细胞状态较为有利。传代时可以直接向培养瓶中添加生长培养基，然后将细胞吹打均匀后移入新的 T25 培养瓶中继续培养。

注意事项：

a) 该细胞对密度较为敏感，培养、传代时请注意保持细胞密度在合适的范围。

b) 首次传代时注意营养，不处理时务必隔天适当补加复苏培养基。

c) FBS 血清需 56°C 加热 30 分钟，可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。

相关产品：

PD-1:PD-L1(B7-H1):PDL2	
Mouse_PDL1 KO LLC1 Cell Line	Mouse_PDL1 KO MC38 Cell Line
aAPC(OKT3) PDL1 CHO-K1 Cell Line	H_PD-1 Reporter Jurkat Cell Line
H_PDCD1LG2(PDL2) aAPC CHO-K1 Cell Line	Mouse PDL1 aAPC CHO-K1 Cell Line
Mouse_PD-1 Reporter Jurkat Cell Line	Canine_PD-1 CHO-K1 Cell Line
Canine_PD-1 HEK-293 Cell Line	Cynomolgus_PD1 CHO-K1 Cell Line
Cynomolgus_PD-L1 HEK-293 Cell Line	H_CD274(PD-L1) CHO-K1 Cell Line
H_CD274(PD-L1) MC38 Cell Line	H_PDCD1(PD-1) CHO-K1 Cell Line
H_PDCD1LG2(PDL2) CHO-K1 Cell Line	H_PDCD1(PD-1) HEK-293 Cell Line
H_PDL1 LLC1(mouse_PDL1 KO) Cell Line	H_PD-L1 HEK-293 Cell Line
H_PDL1 MC38(mouse PDL1 KO) Cell Line	H_PDL1 LLC1(mouse_PDL1 KO) Cell Line
M_PDCD1(PD-1) CHO-K1 Cell Line	H_PD-L1 Raji Cell Line
Anti-Canine_PD1 mIgG2a Antibody(4F12-E6)	Anti-H_CD274(PDL1) hIgG1 Antibody(Atezolizumab)
Anti-H_PDCD1(PD1) hIgG1 Antibody(Budigalimab)	Anti-H_PDCD1LG2 mIgG1 Antibody(3G2)
Anti-H_PDL1 hIgG1 Reference Antibody(Atezbio)	Anti-mouse PD1 RIgG2a Antibody(RMP1-14)
Anti-mouse PD-L1 mIgG1 Antibody(10F.9G2)	Anti-Mouse_PD1 mIgG1 Antibody(29F.1A12)
Anti-mouse_PD1 mIgG1 Antibody(RMP1-14)	Anti-PD1 hIgG4 Antibody(Pembrolizumab)
Anti-PD1 hIgG1 Reference Antibody(Rosnbio)	Anti-PD1 hIgG4 Reference Antibody (Pembio)
Anti-PD1 hIgG4 Reference Antibody (Nivbio)	Anti-PD-1 hIgG4 Reference Antibody (Torbio)
Anti-PD1 hIgG4 Reference Antibody (Sintbio)	Anti-PD-1 hIgG4 Reference Antibody(Tislbio)
Anti-PD1 hIgG4 Reference Antibody(Cambio)	Anti-PDL1 hIgG4 Reference Antibody(Adebio)
Anti-PD-L1 hIgG1 Reference Antibody(Avebio)	Biotinylated Human PDL1 Protein; His-Avi Tag
Anti-PD-L2 hIgG1 Antibody(Hz25G4-1.1)	Cynomolgus PDL1 Protein; His Tag
Biotinylated Human PD1 Protein; His-Avi Tag	Human PD1 Protein; His Tag
Canine PD1 Protein; hFc Tag	Human PDL1 Protein; mFc Tag
Human PD1 Protein; hFc Tag	Mouse PDL1 Protein; His Tag
Human PDL1 Protein; His Tag	Human PDL2 Protein; mFc Tag

使用许可协议：

凡购买及使用本细胞系产品，即表明使用者自愿接受并遵守以下相关使用政策：

- 本细胞系产品限于科研用途，不得被利用于任何商业用途。
- 本产品严禁用于人类或动物疾病诊治，也不得直接用于人体相关实验。
- 用户及为其利益服务的第三方承包商仅可在约定科研范围内使用本材料及其子代，不得进行修饰，亦不得向任何其他实体（包括关联机构）分发、销售、转让或以其他方式提供吉满生物材料。
- 如需将本产品用于本声明范围以外的用途，须事先获得吉满生物科技（上海）有限公司的书面许可，详情请联系吉满生物科技（上海）有限公司。