Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

H_TNFRSF25(DR3) Reporter Jurkat Cell Line

Catalog number: GM-C30290

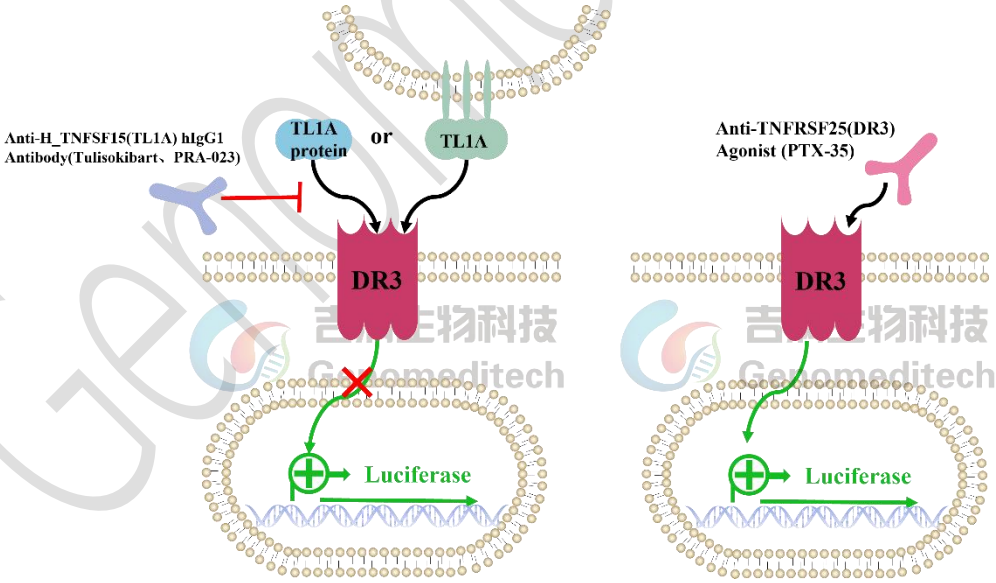
Version 3.3.1.241029

Tumor necrosis factor-like ligand 1A (TL1A), or TNFSF15, is a cytokine primarily expressed by endothelial cells. In T cells, it functions as a co-stimulator, boosting IL-2 reactivity and pro-inflammatory cytokine secretion. TL1A is the sole ligand for death receptor 3 (DR3 or TNFRSF25), a TNF receptor family member that induces apoptosis upon T cell activation. Blocking the TL1A-DR3 interaction is a potential target for chronic immune disease therapies. Furthermore, DR3 agonistic antibodies can reduce regulatory T cell suppression and enhance CD4+ T cell activity in mouse melanoma models, indicating their potential as treatments for solid tumors.

H_TNFRSF25(DR3) Reporter Jurkat Cell Line is a clonal stable Jurkat cell line constitutively expressing the Human TNFRSF25(DR3), along with signal-dependent expression of a luciferase reporter gene. It has two application scenarios:

First, by adding a DR3 agonist drug to activate the downstream signaling pathways in reporter gene cells, and measuring the fluorescence signal to determine the expression of luciferase, it can be used to screen or validate agonist drugs targeting human DR3.

Second, by adding a TL1A antagonist antibody to block the downstream human DR3 signaling activated by TL1A, and measuring the fluorescence signal to determine the expression of luciferase, it can be used to screen or validate antagonist drugs targeting TL1A.



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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	RPMI 1640+10% FBS+1% P.S
Growth medium	RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+0.75 μg/mL Puromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Suspension
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.
RPMI 1640	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
Puromycin	Genomeditech/GM-040401
H_TNFSF15(TL1A) CHO-K1 Cell Line	Genomeditech/GM-C19170
Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35)	Genomeditech/GM-58913AB
Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart、PRA-023)	Genomeditech/GM-58915AB
Human TL1A Protein; His Tag	Genomeditech/GM-84079RP
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

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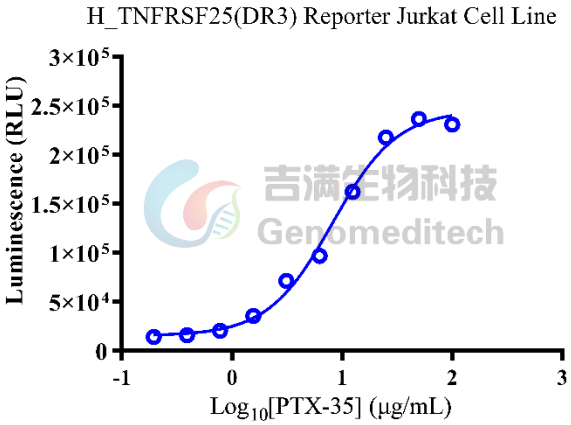
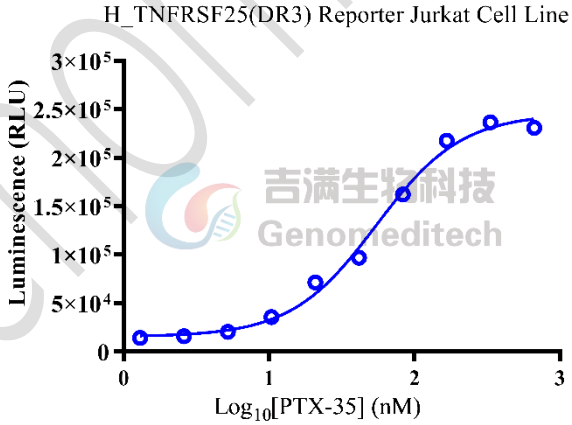
Figures



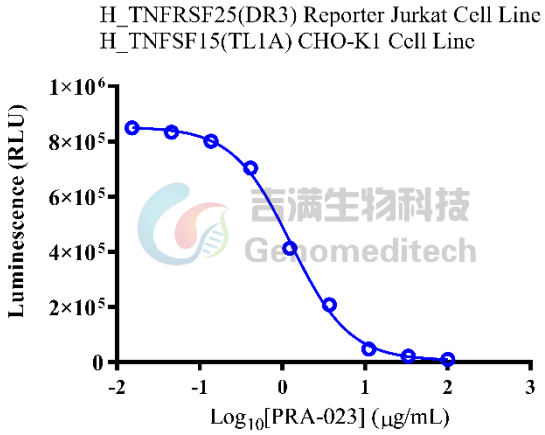
	H_TNFRSF25(DR3) Reporter Jurkat
EC50	8.210

Figure 1 | Response to Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35). The H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35) (Cat. GM-58913AB) in assay buffer (RPMI 1640 + 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 [16.0]. Data are shown by drug mass concentration.



	H_TNFRSF25(DR3) Reporter Jurkat
EC50	54.73

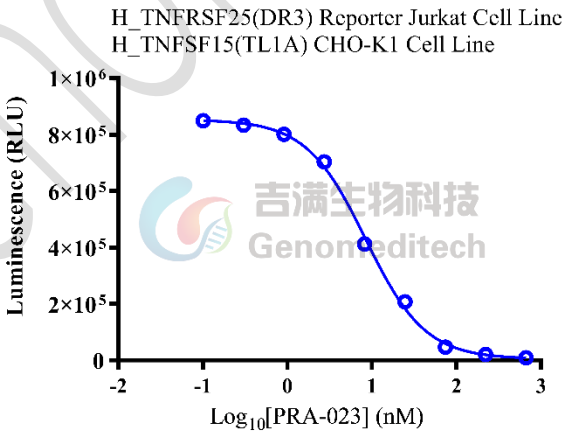
Figure 2 | Response to Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35). The H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35) (Cat. GM-58913AB) in assay buffer (RPMI 1640 + 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 [16.0]. Data are shown by drug molar concentration.



	H_TNFRSF25(DR3) Reporter Jurkat
IC50	1.273

Figure 3 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart、PRA-023)

. Serial dilutions of the Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart、PRA-023)(Cat. GM-58915AB) were incubated with 5E3 cells/well of the H_TNFSF15(TL1A) CHO-K1 Cell Line (Cat. GM-C19170) in a 96-well plate for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Subsequently, the H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a concentration of 1E5 cells/well was added, and the co-culture proceeded for an additional 6 hours. 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 [97.5]. Data are shown by drug mass concentration.

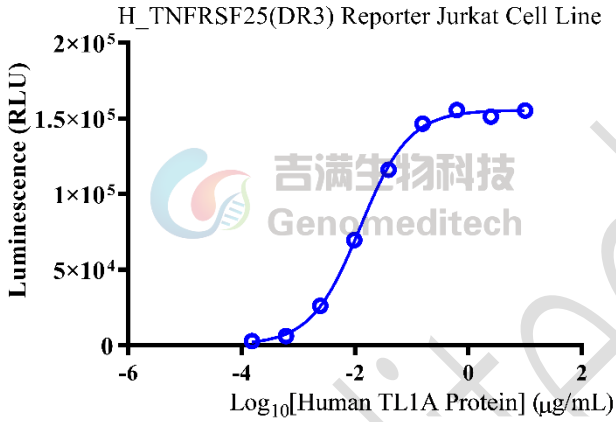


	H_TNFRSF25(DR3) Reporter Jurkat
IC50	8.485

Figure 4 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart、PRA-023)

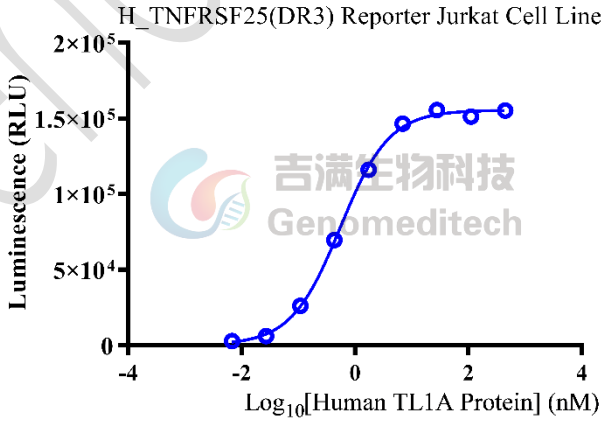
. Serial dilutions of the Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart、PRA-023)(Cat. GM-58915AB) were incubated with 5E3 cells/well of the H_TNFSF15(TL1A) CHO-K1 Cell Line (Cat. GM-C19170) in a 96-well plate for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Subsequently, the H_TNFRSF25(DR3) Reporter Jurkat Cell

Line (Cat. GM-C30290) at a concentration of 1E5 cells/well was added, and the co-culture proceeded for an additional 6 hours. 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 [97.5]. Data are shown by drug molar concentration.



	H_TNFRSF25(DR3) Reporter Jurkat
EC50	0.01236

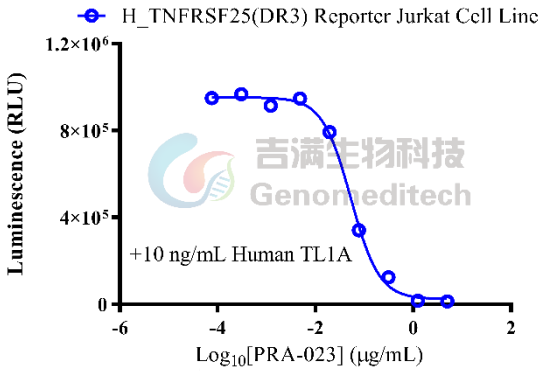
Figure 5 | Response to Human TL1A Protein. The H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Human TL1A Protein (Cat. GM-84079RP) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [55.4]. Data are shown by drug mass concentration.



	H_TNFRSF25(DR3) Reporter Jurkat
EC50	0.5517

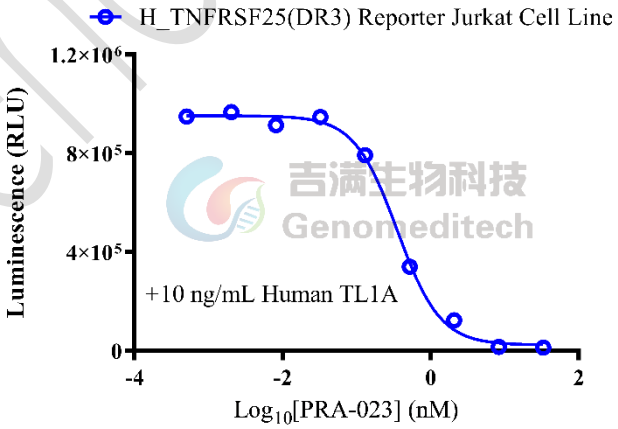
Figure 6 | Response to Human TL1A Protein. The H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Human TL1A Protein (Cat. GM-84079RP) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 6 hours. The firefly luciferase activity was

measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The maximum induction fold was approximately [55.4]. Data are shown by drug molar concentration.



	H_TNFRSF25(DR3) Reporter Jurkat
IC50	0.05321

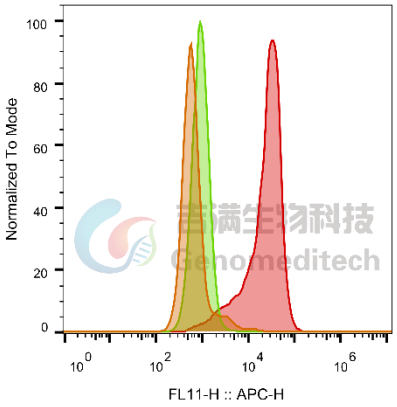
Figure 7 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart 、 PRA-023). Prepare the H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a density of 1E5 cells/well in a 96-well plate. Incubate serial dilutions of Anti-H_TNFSF15(TL1A) hIgG1 Antibody (Tulisokibart, PRA-023) (Cat. GM-58915AB) with 10 ng/mL Human TL1A Protein (Cat. GM-84079RP) for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Add the mixture to the Jurkat cells and incubate for 6 hours. Measure Firefly luciferase activity using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Results showed a maximum blocking fold of approximately 73.5, with data presented by drug mass concentration.



	H_TNFRSF25(DR3) Reporter Jurkat
IC50	0.3547

Figure 8 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart 、 PRA-023). Prepare the H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a density of 1E5 cells/well in a 96-well plate. Incubate serial dilutions of Anti-H_TNFSF15(TL1A) hIgG1 Antibody (Tulisokibart, PRA-023) (Cat. GM-58915AB)

with 10 ng/mL Human TL1A Protein (Cat. GM-84079RP) for 1 hour in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). Add the mixture to the Jurkat cells and incubate for 6 hours. Measure Firefly luciferase activity using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Results showed a maximum blocking fold of approximately 73.5, with data presented by drug molar concentration.



SampleID	Geometric Mean : FL11-H
Jurkat Anti-DR3+APC-2nd Ab	661
Jurkat H_TNFRSF25(DR3) H_IgG+APC-2nd Ab	937
Jurkat H_TNFRSF25(DR3) Anti-DR3+APC-2nd Ab	20985

Figure 9 | H_TNFRSF25(DR3) Reporter Jurkat Cell Line was determined by flow cytometry using Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35) (Cat. GM-58913AB).

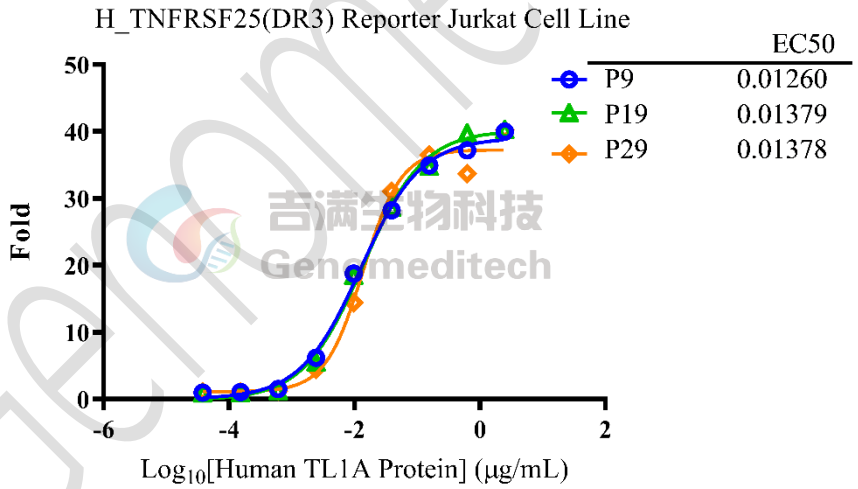


Figure 10 | The passage stability of response to Human TL1A Protein. The passage 9,19 and 29 of H_TNFRSF25(DR3) Reporter Jurkat Cell Line (Cat. GM-C30290) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Human TL1A Protein (Cat. GM-84079RP) in assay buffer (RPMI 1640 + 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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Cell Recovery

Recovery Medium: RPMI 1640+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 complete medium. And dispense the suspension into 1 - 2 T-25 culture flasks.
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: RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+0.75 μg/mL Puromycin

Approximately 48-72 hours after the initial thawing, the cells can be passaged for the first time. After this initial passage, the culture medium can be adjusted to growth medium supplemented with antibiotics. If cells are not passaged within 48 hours, it is recommended to add some fresh recovery medium and place the flask horizontally.

a) When the cell density reaches 1.5 - 2E6 cells/mL, subculture the cells. Do not allow the cell density to exceed 2E6 cells/mL.
b) It is recommended to use T-25 flasks for subculturing.
c) These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.
d) During passaging, you can directly add fresh growth medium to the culture flask, gently pipette to resuspend the cells, and then transfer the cell suspension to a new T-25 flask for continued culture.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 3E5 and 1E6 viable cells/mL.

Medium Renewal: Every 2 to 3 days

Notes

a) These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.

b) During the first passage, pay attention to the nutrient supply; if not subculturing, make sure to add fresh recovery medium every other day as needed.

Related Products

IL-23	
H_IL-23 Reporter 293 Cell Line	
TNF:TNFR2:TNFR1	
H_TNFR2 Null Reporter Cell Line	H_TNFR2 Reporter Jurkat Cell Line
H_TNFR2 Reporter V2 Cell Line	Cynomolgus_TNFRSF1B(TNFR2) CHO-K1 Cell Line
H_TNFRSF1B(TNFR2) CHO-K1 Cell Line	H_TNFRSF1B(TNFR2) HEK-293 Cell Line
Membrane Bound H_TNFα CHO-K1 Cell Line	Membrane Bound H_TNFα(cleavage-resistant) CHO-K1 Cell Line
Anti-H_TNFR2 hIgG1 Antibody(1H10)	Anti-H_TNFRSF1B(TNFR2) hIgG1 Antibody(UC2.3.8)
Anti-TNFR1 hIgG1 Antibody(Atrosab)	Anti-TNF-α hIgG1 Antibody（CT-P17）
TL1A:DR3(TNFRSF25)	
H_TNFSF15(TL1A) Reporter Cell Line	Mouse_TNFRSF25(DR3) Reporter Jurkat Cell Line
Cynomolgus_TNFSF15(TL1A) HEK-293 Cell Line	H_TNFRSF25(DR3) CHO-K1 Cell Line
H_TNFRSF25(DR3) HEK-293 Cell Line	H_TNFSF15(TL1A) CHO-K1 Cell Line
H_TNFSF15(TL1A) HEK-293 Cell Line	Mouse_TNFSF15(TL1A) HEK-293 Cell Line
Anti-H_TNFSF15(TL1A) hIgG1 Antibody(PF-06480605)	Anti-H_TNFSF15(TL1A) hIgG1 Antibody(Tulisokibart、PRA-023)
Anti-H_TNFSF15(TL1A) hIgG4 Antibody	Anti-TL1A hIgG1 Reference Antibody (Duvbio)
Anti-TL1A hIgG1 Reference Antibody (Tulbio)	Anti-TNFRSF25(DR3) hIgG1 Antibody(PTX-35)
Cynomolgus TL1A Protein; His Tag	Human TL1A Protein; His Tag

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