Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

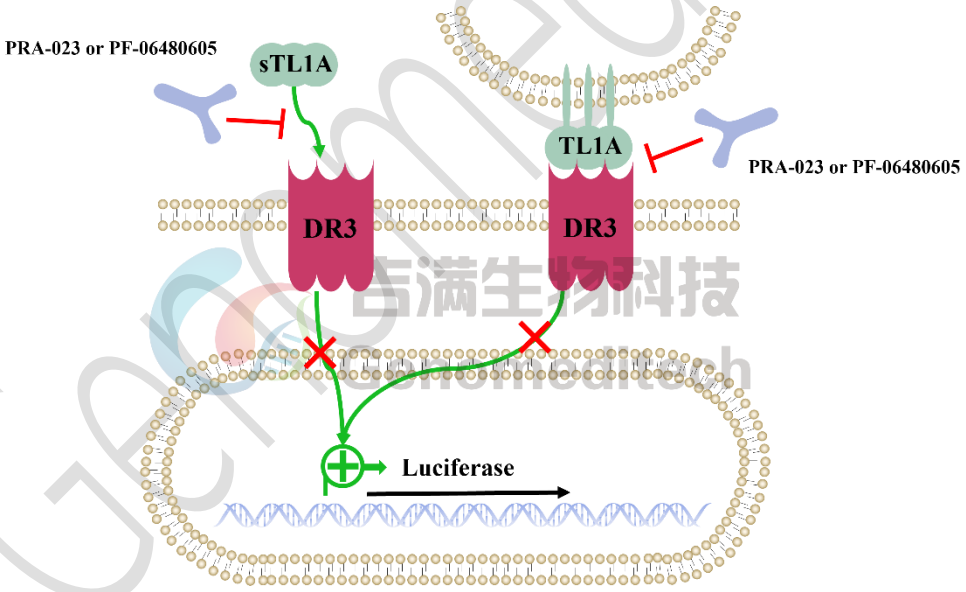
H_TNFSF15(TL1A) Reporter Cell Line

Catalog number: GM-C30289

Version 3.3.1.241108

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_TNFSF15(TL1A) Reporter Cell Line is a clonal cell line that expresses human DR3 endogenously, along with signal-dependent expression of a luciferase reporter gene. When TL1A binds to DR3, it activates downstream signaling pathways, leading to the expression of luciferase. Blockade antibodies can block this signal transmission. The luciferase activity measurement indicates the activation level of the signaling pathway and can thus be used to evaluate the in vitro effects of a neutralizing antibody targeting TL1A.



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Specifications

Quantity	3E6 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+2 ng/mL GM-CSF
Growth medium	RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF+3 μg/mL Blasticidin
Note	None
Freezing Medium	90% FBS+10%DMSO
Growth properties	Mixed: suspension with some adherent cells
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.
Blasticidin	Genomeditech/GM-040404
Recombinant Human GM-CSF	Novoprotein/C003
Pen/Strep	Thermo/15140-122
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
RPMI 1640	gibco/C11875500BT
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
Anti-H_TNFSF15(TL1A) hIgG1 Antibody(PF-06480605)	Genomeditech/GM-59479AB
Human TL1A Protein; His Tag	Genomeditech/GM-84079RP
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

Figures

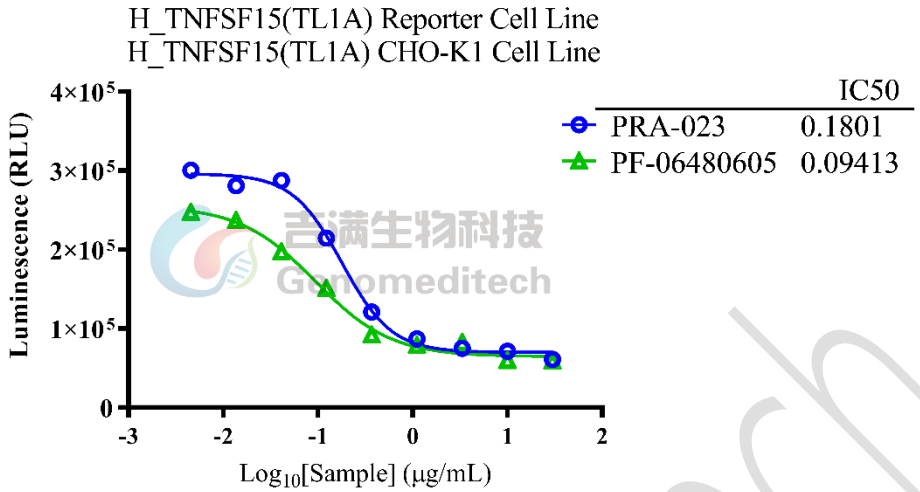


Figure 1 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody (Tulisokibart、PRA-023) and Anti-H_TNFSF15(TL1A) hIgG1 Antibody (PF-06480605). Serial dilutions of the PRA-023, PF-06480605 was incubated with 1E4 cells/well of the H_TNFSF15(TL1A) CHO-K1 Cell Line (Cat. GM-C19170) in a 96-well plate for 1 hour. Subsequently, the H_TNFSF15(TL1A) Reporter Cell Line (Cat. GM-C30289) at 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). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [4.6] and [4.3], respectively. Data are shown by drug mass concentration.

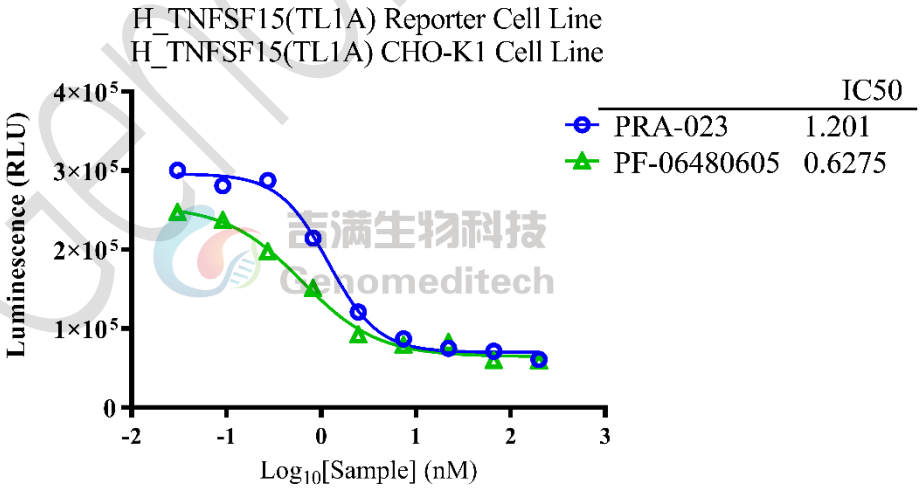
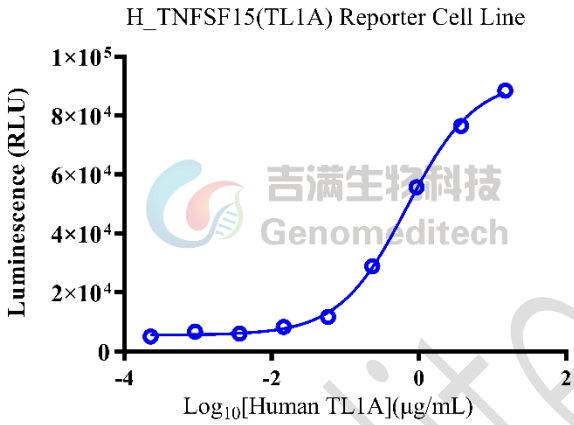


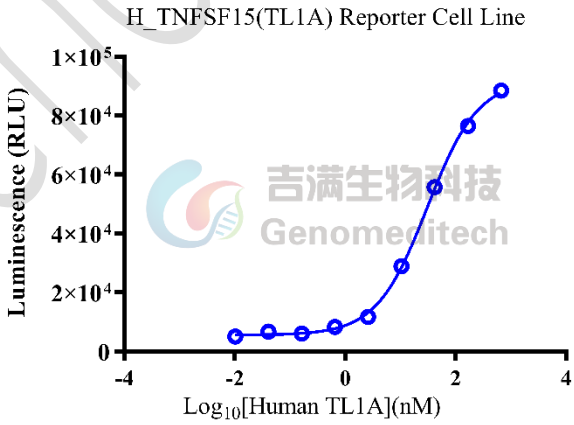
Figure 2 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody (Tulisokibart、PRA-023) and Anti-H_TNFSF15(TL1A) hIgG1 Antibody (PF-06480605). Serial dilutions of the PRA-023, PF-06480605 was incubated with 1E4 cells/well of the H_TNFSF15(TL1A) CHO-K1 Cell Line (Cat. GM-C19170) in a 96-well plate for 1 hour. Subsequently, the H_TNFSF15(TL1A) Reporter Cell Line (Cat. GM-C30289) at 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). Firefly luciferase activity is then measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). The results indicated maximum blocking folds of approximately [4.6] and [4.3], respectively. Data are shown by drug molar concentration.



	H_TNFSF15(TL1A) Reporter
EC50	0.6897

Figure 3 | Response to Human TL1A Protein; His Tag. H_TNFSF15(TL1A) Reporter Cell Line (Cat. GM-C30289) at a concentration of 4E4 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 [12.6]. Data are shown by drug mass concentration.



	H_TNFSF15(TL1A) Reporter
EC50	30.79

Figure 4 | Response to Human TL1A Protein; His Tag. H_TNFSF15(TL1A) Reporter Cell Line (Cat. GM-C30289) at a concentration of 4E4 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 [12.6]. Data are shown by drug molar concentration.

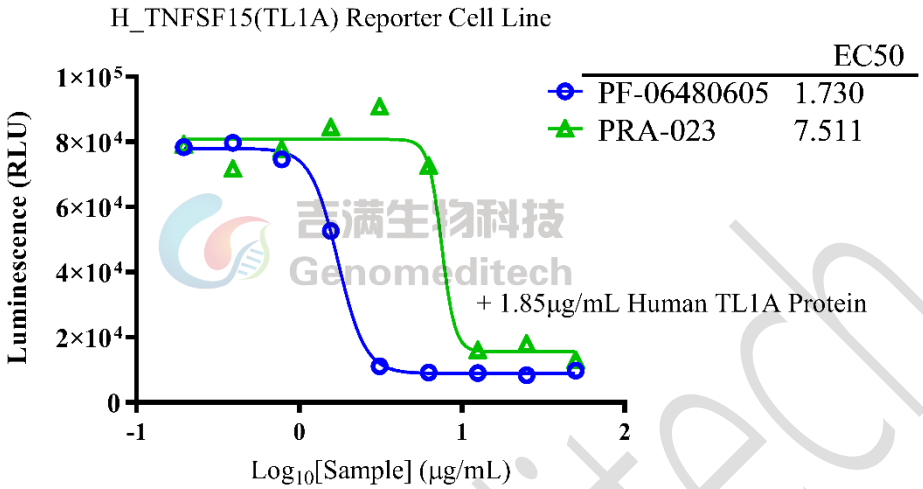


Figure 5 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody (Tulisokibart、PRA-023) and Anti-H_TNFSF15(TL1A) hIgG1 Antibody (PF-06480605). The serial dilutions of PRA-023, PF-06480605 were incubated with 1.85 μg/mL of Human TL1A Protein (Cat. GM-84079RP) for 1 hour. After pre-incubation, add the mixture to the H_TNFSF15(TL1A) reporter cell line at a density of 4E4 cells/well in a 96-well format, and incubate for 6 hours in assay buffer (RPMI 1640 + 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 maximum blocking folds of approximately [8.8] and [6.8], respectively. Data are shown by drug mass concentration.

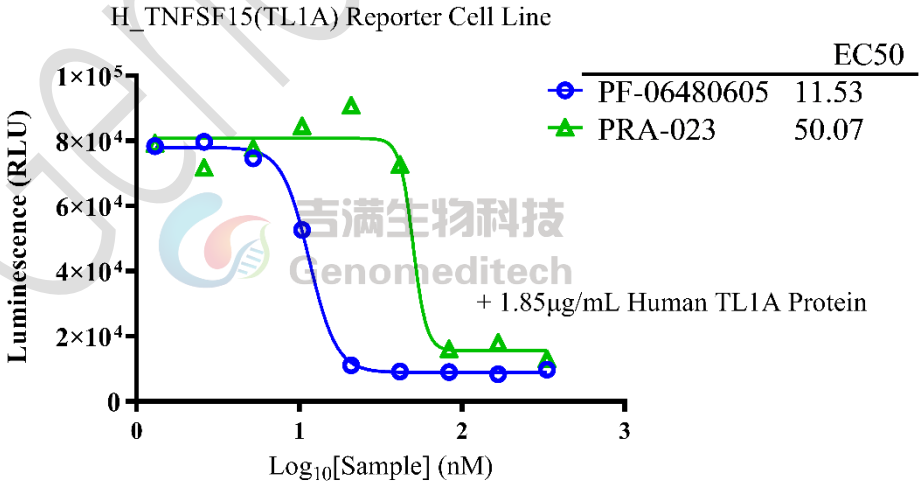


Figure 6 | Response to Anti-H_TNFSF15(TL1A) hIgG1 Antibody (Tulisokibart、PRA-023) and Anti-H_TNFSF15(TL1A) hIgG1 Antibody (PF-06480605). The serial dilutions of PRA-023, PF-06480605 were incubated with 1.85 μg/mL of Human TL1A Protein (Cat. GM-84079RP) for 1 hour. After pre-incubation, add the Anti-H_TNFSF15(TL1A)

mixture to the H_TNFSF15(TL1A) reporter cell line at a density of 4E4 cells/well in a 96-well format, and incubate for 6 hours in assay buffer (RPMI 1640 + 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 maximum blocking folds of approximately [8.8] and [6.8], respectively. Data are shown by drug molar concentration.

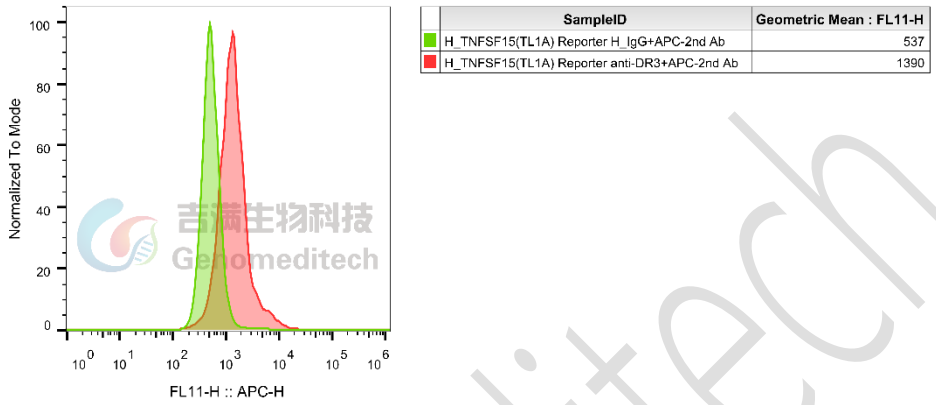


Figure 7 | H_TNFSF15(TL1A) Reporter Cell Line was determined by flow cytometry using Anti-H_DR3 hIgG1 Antibody(PTX-35) (Cat. GM-58913AB).

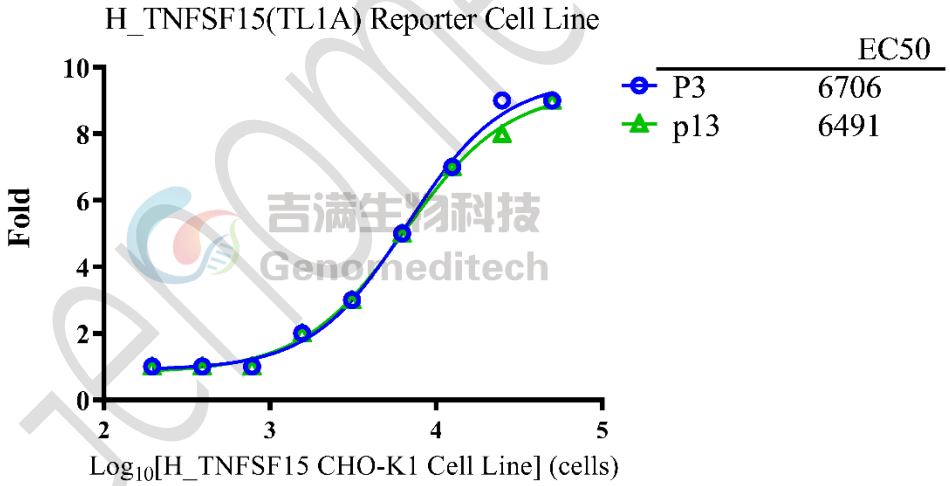


Figure 8 | Response to H_TNFSF15 CHO-K1 Cell Line. The passage 3,13 of H_TNFSF15(TL1A) Reporter Cell Line (Cat. GM-C30289) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of H_TNFSF15(TL1A) CHO-K1 Cell Line (Cat. GM-C19170) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 7 hours. The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503).

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Cell Recovery

Recovery Medium: RPMI 1640+10% FBS+1% P.S+2 ng/mL GM-CSF

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 the cell pellet using the recommended complete medium and adjust the viable cell density to 4-6E5 cells/mL. Then dispense the suspension into an appropriate culture flask and initially place the flask in an upright position after thawing.

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 3E6 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+2 ng/mL GM-CSF+3 μg/mL Blasticidin

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.

a) This cell is a human erythroid leukemia cell, lymphoblast, growing in suspension.

b) In the suspension, they appear as large, single, round cells. Cells shed a large accumulation of cytoplasmic granules in the culture, which should not be confused with bacteria!

c) When the cell density reaches 1-1.2E6 cells/mL, perform a 1:2 to 1:3 split, ensuring subculturing every other day. It is essential to perform a full-volume centrifugation and medium replacement during passaging. Do not let the density exceed 1.2E6 cells/mL. It is recommended to use T-25 flasks for subculturing, and you can control the cell density for subculturing by counting.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 4E5 and 6E5 viable cells/mL.

Medium Renewal: Every other day

Notes

a) To minimize the presence of cytoplasmic granules, it is essential to passage the cells every other day when the cell density reaches 1-1.2E6 cells/mL. During passaging, perform a complete centrifugation and replace the culture medium to ensure appropriate cell density and cytokine concentration. Failure to do so may promote the growth of factor-independent subclones.

Related Products

IL-23	
H_IL-23 Reporter 293 Cell Line	H_IL-23R HEK-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_TNFRSF25(DR3) Reporter Jurkat 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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