

1. Flow-through equipment operation process: QC → single dye compensation → sample → analysis.

2. Flow-through circle gates and compensation options:

For a selection of circle doors see the Supplementary Materials 2.

① cell membrane:

Gate	Count	% Parent	% Grandparent
All	356,935		
└─ P1	26,508	7.43%	
└─ P10	23,788	89.74%	6.66%
└─ M11	16,256	68.34%	61.32%
└─ V51+	504	3.10%	2.12%
└─ CD27-V51+	48	9.52%	0.30%
└─ CD27+V51+	35	6.94%	0.22%
└─ CD69-CD25+V51+	3	0.60%	0.02%
└─ CD69+CD25+V51+	1	0.20%	0.01%
└─ CD69-CD25-V51+	437	86.71%	2.69%
└─ CD69+CD25-V51+	63	12.50%	0.39%
└─ CD86-CD80+V51+	4	0.79%	0.02%
└─ CD86+CD80+V51+	0	0.00%	0.00%
└─ CD86-CD80-V51+	500	99.21%	3.08%
└─ CD86+CD80-V51+	0	0.00%	0.00%
└─ Q3-2	1	0.01%	0.00%
└─ Q3-3	15,466	95.14%	65.02%
└─ V52+	285	1.75%	1.20%
└─ CD86-CD80+V52+	0	0.00%	0.00%
└─ CD86+CD80+V52+	0	0.00%	0.00%
└─ CD86-CD80-V52+	285	100.00%	1.75%
└─ CD86+CD80-V52+	0	0.00%	0.00%
└─ CD27-V52+	188	65.96%	1.16%
└─ CD27+V52+	87	30.53%	0.54%
└─ CD69-CD25+V52+	2	0.70%	0.01%
└─ CD69+CD25+V52+	0	0.00%	0.00%
└─ CD69-CD25-V52+	261	91.58%	1.61%
└─ CD69+CD25-V52+	22	7.72%	0.14%

Circle doors: AII→SSC/FSC (P1) →Single cell (P10) →CD3 (M11) →Vδ1/ Vδ2

Table 1 Compensation Matrix and Overflow matrix

Source/objective	Vδ1-FITC	CD3-PerCP-eFluor710	Vδ2-PE-Cy7	CD25-APC	CD69-APC-Cy7	CD80-Pacific	CD27-BV510	CD86-BV650
Vδ1-FITC	-100.0068	0.3839	-0.2211	-0.0094	-0.0764	-0.0981	4.0700	-0.3733
CD3-PerCP-eFluor 710	-0.0017	-101.5909	58.5246	10.4814	22.1606	-0.7718	0.0846	25.4960
Vδ2-PE-Cy7	0.0000	2.3601	-102.2613	-0.4069	9.8280	0.0279	0.0200	-0.5771
CD25-APC	-0.0005	0.4251	-1.1173	-101.1737	11.9624	-0.2655	0.0131	8.3475
CD69-APC-Cy7	-0.0001	-0.1673	8.9095	1.7035	-101.0694	0.0332	0.0669	0.0191
CD80-Pacific Blue	-0.0375	0.0123	-0.0065	0.2107	0.0502	-100.6976	22.3760	-1.9134
CD27-BV510	0.1688	-0.0609	0.0313	-1.0286	-0.2371	3.1127	-100.7997	9.4829
CD86-BV650	0.0057	0.6426	-0.3292	10.9852	2.5273	3.4634	0.3171	-101.1264

Table 2

Detector gain-NC							
Vδ1-FITC	CD3-PerCP-eFluor710	Vδ2-PE-Cy7	CD25-APC	CD69-APC-Cy7	yδTCR-Pacific Blue	CD27-BV510	CD86-BV650
453	617	545	551	545	378	453	551

②intracellular

Table 3 Compensation Matrix and Overflow matrix

Source/objective	Vδ1-FITC	TNFα-PE	CD3-PerCP-Cy5.5	Vδ2-PE-Cy7	IFNγ--APC	IL17A-BV421	CD27-BV510
Vδ1-FITC	-100.4761	10.3806	-0.3439	-0.1332	0.0492	-0.0993	3.9806
TNFα-PE	4.5412	-100.4821	6.9399	2.6051	-1.0093	0.0100	0.3588
CD3-PerCP-Cy5.5	0.0017	-0.0360	-100.3018	11.4640	14.7726	0.1561	-0.0465
Vδ2-PE-Cy7	-0.0205	0.4512	2.0150	-100.2456	-0.2822	0.0102	0.0360
IFNγ-APC	0.0000	-0.0008	0.4737	0.1612	-100.0698	0.0175	0.0472
IL17A-BV421	-0.0388	-0.0039	0.0004	0.0002	-0.0009	-100.7477	22.3167
CD27-BV510	0.1750	0.0176	-0.0019	-0.0007	0.0042	3.3756	-100.7548

Table 4

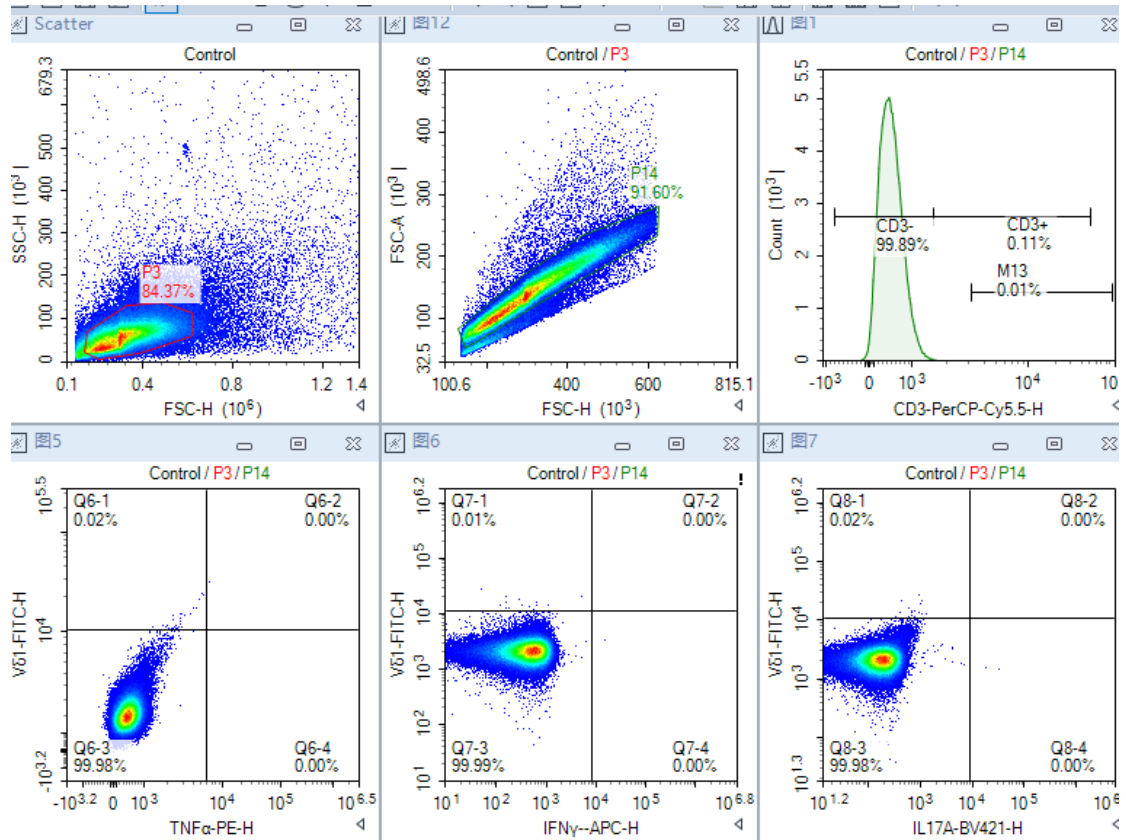
Detector gain- Control						
V δ 1-FITC	TNF α -PE	CD3-PerCP-Cy5.5	V δ 2-PE-Cy7	IFN γ --APC	IL17A-BV421	CD27-BV510
453	402	617	545	551	378	453

Gate	Count	% Parent	% Grandparent
All	129,040		
└─ P3	112,112	86.88%	
└─ P14	104,327	93.06%	80.85%
└─ CD3-	34,529	33.10%	30.80%
└─ CD3+	69,374	66.50%	61.88%
└─ Vδ1	1,274	1.84%	1.22%
└─ Q4-2	3	0.00%	0.00%
└─ Q4-3	67,022	96.61%	64.24%
└─ Vδ2	1,075	1.55%	1.03%
└─ Q5-1	1,277	1.84%	1.22%
└─ Q5-2	0	0.00%	0.00%
└─ Q5-3	67,837	97.78%	65.02%
└─ Q5-4	260	0.37%	0.25%
└─ Q6-1	200	0.29%	0.19%
└─ Q6-2	1,082	1.56%	1.04%
└─ Q6-3	6,804	9.81%	6.52%
└─ Q6-4	61,288	88.34%	58.75%
└─ Q7-1	1,179	1.70%	1.13%
└─ Q7-2	44	0.06%	0.04%
└─ Q7-3	46,835	67.51%	44.89%
└─ Q7-4	21,316	30.73%	20.43%
└─ Q8-1	1,255	1.81%	1.20%
└─ Q8-2	0	0.00%	0.00%
└─ Q8-3	68,005	98.03%	65.18%
└─ Q8-4	114	0.16%	0.11%
└─ Q9-1	1,124	1.62%	1.08%
└─ Q9-2	0	0.00%	0.00%
└─ Q9-3	68,240	98.37%	65.41%
└─ Q9-4	10	0.01%	0.01%
└─ Q10-1	80	0.12%	0.08%
└─ Q10-2	1,021	1.47%	0.98%
└─ Q10-3	8,035	11.58%	7.70%
└─ Q10-4	60,238	86.83%	57.74%
└─ Q11-1	756	1.09%	0.72%
└─ Q11-2	368	0.53%	0.35%
└─ Q11-3	45,817	66.04%	43.92%
└─ Q11-4	22,433	32.34%	21.50%
└─ Q12-1	968	1.40%	0.93%
└─ Q12-2	0	0.00%	0.00%

Circle doors: All→SSC/FSC (P3) →Single cell (P14) →CD3→Vδ1/ Vδ2

3. Blank controls

Control is Blank controls



4. Cell count status

cell membrane: 1×10^6

intracellular: 5×10^6