

SOP, CAR-T Immunophenotyping

Materials & Equipment:

1.0. Media and Reagents

- 1.0. 0.4% Trypan Blue Stain
- 1.1. 4% PFA
- 1.2. Conjugated Antibodies
- 1.3. DPBS without Ca²⁺, Mg²⁺
- 1.4. FACS Sheath Fluid Buffer
- 1.5. Fetal Bovine Serum (FBS)

2.0. Consumables

- 2.1. Centrifuge Tubes (15 mL)
- 2.2. BD Round Bottom Tubes
- 2.3. Microcentrifuge Tubes (1.5 mL)
- 2.4. Countess Cell Counting Chamber Slide

3.0. Equipment

- 3.1. BD Flow Cytometry System FACS Calibur
- 3.2. TOMY Refrigerated Centrifuge-5810R
- 3.3. GRANT Mini Vortex Mixer PV-1
- 3.4. OLYMPUS Research Inverted Microscope
- 3.5. SAMSUNG Refrigerator RL40EGPS

Procedure:

- 1.1. Prepare FACS Buffer by adding PBS with 2% FBS.
- 1.2. Centrifuge the cells obtained from Manufacturing department at 400 x g for 5 minutes.
- 1.3. Remove the supernatant and re-suspend the cells by adding in 10 mL DPBS.
- 1.4. Determine the cell count.
- 1.5. Transfer 1.0×10^6 cells into twelve round bottom tubes.
- 1.6. Centrifuge the cells at 400 x g for 5 minutes.
- 1.7. Remove the supernatant.
- 1.8. Re-suspend the pellet in 1000 μ L of FACS Buffer.
- 1.9. To block non-specific Fc-mediated interaction, add 2.5ug of Fc Block per 10^6 cells per tube and incubate for 10 minutes at room temperature.
- 1.10. Centrifuge the cells at 400 x g for 5 minutes.
- 1.11. Re-suspend the pellet in 100 μ L of FACS Buffer.
- 1.12. Prepare antibodies cocktail as the table below into separated 1.5ml microcentrifuge tubes.
- 1.13. Add 100 μ L of cells suspension into antibody cocktail.
- 1.14. Incubate for 20 minutes at 4°C in the dark (cover tube with foil).
- 1.15. Top-up with additional 1000 μ l of FACS Buffer.
- 1.16. Spin tube at 300 x g for 5 minutes at 4°C.
- 1.17. Resuspend the pellet with 500 μ l FACS Buffer.
- 1.18. Cover with foil and place tube on ice.
- 1.19. Acquire at least 10,000 events on a BD FACSCalibur.

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Type	Tube	Marker	Volume (µL)
Control	1	Unstained	nil
	2	CD 4 PE	20
	3	CD 8 PerCP-Cy5.5	5
	4	CD 3 APC	20
	5	CD 19 CAR green	2
Isotype Control	6	FITC LABELED MOUSE IGG1, KPA ITCL (MAB555748)	20
		PE LABELED MOUSE IGG1, KAPPA I/CTRL (MAB555749)	20
		PerCP-CY5.5 mouse IgG1 isotype control (550795)	20
		MS IGG1 KPA ITCL APC MAB MOPC-21 (MAB555751)	20
FMO CD4 PE	7	CD 8 PerCP-Cy5.5	20
		CD 3 APC	20
		CD 19 CAR green	2
FMO CD8 PerCP-Cy5.5	8	CD 4 PE	20
		CD 3 APC	20
		CD 19 CAR green	2
FMO CD 3 FITC	9	CD 4 PE	20
		CD 8 PerCP-Cy5.5	20
		CD 19 CAR green	2
FMO CD19 CAR green	10	CD 4 PE	20
		CD 8 PerCP-Cy5.5	20
		CD 3 APC	20
Sample	11	CD4 PE	20
		CD8 PERCP	20
		CD 3 APC	20
		CD19 CAR green	2
	12	7AAD	5