Flow Cytometry protocol for Human Immune System Engrafted Precision Research Models

1. PURPOSE

a. This protocol covers the process of flow cytometry immunostaining for assessment of human cell reconstitution (i.e. engraftment efficiency) in human immune system engrafted precision research models.

2. Reagents used

Reagent	Vendor	Catalog Number
10X Phosphate Buffered Saline (PBS)	VWR	101076-194
Fetal Bovine Serum (FBS)	Life Technology	10438-026
Sodium Azide	Sigma	S2002
FACS Lysing Solution	BD Bioscience	349202
Stabilizing Fixative	BD Bioscience	338036
Dimethyl Sulfoxide (DMSO)	Sigma	D2650

3. Antibody information

Antibody	Antibody Clone	Fluorescent dye conjugate	Vendor	Catalog Number
mCD45	30-F11	PerCP-Cy5.5	BioLegend	103132
hCD45	HI30	PE	BioLegend	304039
hCD3	UCHT1	APC	BioLegend	300439
hCD20	2H7	APC-Cy7	BioLegend	302314

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Flow Cytometry Staining Procedures

****IMPORTANT: KEEP All SAMPLES ON ICE THROUGHOUT PROCEDURE**

1. Prepare 100 ul volume of anticoagulated whole blood and place samples on ice
2. Prepare antibody dilutions according to manufacturer's recommendations. To ensure proper performance, **It is recommended that antibody reagents be titrated and optimized for use in your own facility. For further information on antibody staining, test validations, and cytometer set-up, consult with antibody manufacturer and/or cytometer manufacturer.**
3. Add the antibody mix to the appropriate sample tubes.
4. Add 90µl of whole blood specimen in EDTA to the labeled tubes.
5. Mix and incubate in the dark on ice for 40 min.
6. Add 2 ml RBC lysis solution to each tube. Brief vortex, then incubate on ice for 10 min in the dark.
7. Spin for 5 min at 1100rpm (200x g) and remove supernatant.
8. Add 1 ml standard wash buffer to each tube and vortex.
9. Spin 5 min at 1100 rpm (200x g) and remove supernatant.
10. Add 500 µl stabilizing fixative working solution to each tube, gently vortex and incubate 20 min in the dark.
11. Spin 5 min at 1100 rpm (200x g).
12. Remove supernatant carefully.
13. Wash 2X by adding 1ml standard wash buffer, gently vortex, spin at 1100 rpm for 5 min and remove supernatant.
14. After final wash and supernatant removed, re-suspend cells with 500 µl standard wash buffer, mix, and transfer the suspension to labeled polystyrene tubes.
15. Analyze samples on flow cytometer.

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Analysis of flow cytometry data

Gating Strategy

1. Gate events by plotting SSC-A on y-axis and FSC-H on x-axis.
    a. Draw gate to include lymphocytes and granulocytes.
    b. Plot gated events by mouse CD45 vs human CD45.
        i. The % hCD45+ cells are representative of immune system reconstitution.
    c. Sub-gate on human CD45+ cells
        i. Plot hCD3 vs hCD20 to evaluate relative percent of T and B cells respectively.