Standard protocols for immune profiling of peripheral blood leucocyte subsets by flow cytometry using DuraClone IM reagents

Katharina Kronenberg
Department of Surgery, University Hospital Regensburg, Germany
Paloma Riquelme (Paloma.Riquelme@ukr.de)
Department of Surgery, University Hospital Regensburg, Germany https://orcid.org/0000-0001-8962-3156
James A. Hutchinson
Department of Surgery, University Hospital Regensburg, Germany https://orcid.org/0000-0002-1199-4210

Method Article

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Abstract

This document describes standard protocols used by the Hutchinson group for profiling human peripheral blood leucocytes by flow cytometry analyses using DuraClone IM reagents.

Introduction

This document describes standard protocols used by the Hutchinson group for analysis of human blood leucocyte populations by flow cytometry (1). DuraClone reagents are dry pre-formulated antibody panels for extensive phenotyping of T cells, B cells, NK cells, dendritic cells and granulocytes in whole blood. In our experience, DuraClone IM Tubes are a convenient, reliable and highly standardised option for immune monitoring of clinical trials. In part, these protocols were adapted from the publications of Streitz, Kverenland and colleagues (2-3), as well as manufacturer's recommendations issued by Beckman Coulter.

Reagents

Reagents

DuraClone IM Phenotyping Basic Tube (B53309, Beckman Coulter)

DuraClone IM T cell subsets Tube (B53328, Beckman Coulter)

DuraClone IM TCRs Tube (B53340, Beckman Coulter)

DuraClone IM Granulocytes Tube (B88651, Beckman Coulter)

DuraClone IM Dendritic Cells Tube (B53351, Beckman Coulter)

DuraClone IM B cells Tube (B53318, Beckman Coulter)

DuraClone IM Treg Tube (B53346, Beckman Coulter)

VersaLyse Solution (A09777, Beckman Coulter)

DPBS without Ca^{2+} or Mg^{2+} (D8537, Sigma)

IOTest 3 Fixative Solution (10x) (A07800, Beckman Coulter)

FBS South American (10270106, Thermo Fisher), heat-inactivated

Ecotainer Aqua B, Distilled water (0082423E, Braun)

PerFix-nc Kit (B31168, Beckman Coulter)

Materials

S-Monovette® 2,7 ml, K3 EDTA (05.1167, Sarstedt)

15 ml tube, PP, 17/120 mm, conical bottom (188271, Greiner Bio-One)

Equipment

Equipment

Navios cytometer: Standard configuration with 10 colours, 3 lasers (Beckman Coulter)

Cytometry List Mode Data Acquisition and Analysis Software (Beckman Coulter)

Kaluza analysis software v.1.3 (Beckman Coulter)

Procedure

1. Preparation of reagents

1.1 Preparation of 0.8% IOTest 3 Fixative Solution

1. Dilute 100 µl IOTest 3 Fixative Solution (10X) with 900 µl PBS to obtain 0.8 % IOTest 3 Fixative Solution

1.2 Preparation of 0.1% IOTest 3 Fixative Solution

1. Dilute 12.5 µl IOTest 3 Fixative Solution (10X) with 987.5 µl PBS to obtain 0.1 % IOTest 3 Fixative Solution

1.3 Preparation of 1X PerFix-nc Buffer 3

1. Prepare a 1/10 dilution of 10X Buffer 3 in dd$H_2$0: dilute 1 part 10X Buffer 3 with 9 parts of ddH_2O

2. Sample preparation using DuraClone IM Tubes: Phenotyping Basic, T cell subsets, TCRs and Dendritic Cells

1. Collect peripheral blood samples directly into EDTA-vacutainers

2. Samples can be stored at 4 °C for a maximum of 4 h before processing

3. Mix the blood sample by repeatedly inverting the vacutainer

4.

4.1. For DuraClone IM Phenotyping Basic, T cell, TCRs and Granulocytes Tube: transfer 100 µl blood into the DuraClone Tube

4.2. For DuraClone IM Dendritic Cells Tube: transfer 200 µl blood into the DuraClone Tube

5. Vortex tube for 8 seconds

6. Incubate at room temperature in the dark for 15 min

7. Add 2 ml VersaLyse Solution

8. Vortex tube for 3 seconds

9. Incubate at room temperature in the dark for 15 min

10. Pellet cells by centrifugation at 200 g for 5 min

11. Aspirate supernatant using a vacuum pump

12. Vortex tube for 3 seconds

13. Add 3 ml DPBS

14. Pellet cells by centrifugation at 200 g for 5 min

15. Aspirate supernatant using a vacuum pump

16. Resuspend cell pellet in 380 µl 0.8 % IOTest 3 Fixative Solution for data collection using a Navios flow cytometer

3. Sample preparation using DuraClone IM Tubes: B cells

1. Collect peripheral blood samples directly into EDTA-vacutainers

2. Samples can be stored at 4 °C for a maximum of 4 h before processing

3. Mix the blood sample by repeatedly inverting the vacutainer

4. Transfer 10 ml DPBS into a 15 ml conical bottom tube
5. Add 300 µl blood to the 10 ml DPBS
6. Mix by inverting the tube
7. Pellet cells by centrifugation at 300 g for 10 min
8. Aspirate supernatant using a vacuum pump
9. Vortex tube for 3 seconds
10. Add 10 ml DPBS
11. Mix by inverting the tube
12. Pellet cells by centrifugation at 300 g for 5 min
13. Aspirate supernatant using a vacuum pump
14. Resuspend cell pellet in DPBS to get a final volume of 300 µl
15. Transfer 100 µl of washed blood sample into the DuraClone IM B cells Tube
16. Vortex tube for 8 seconds
17. Incubate at room temperature in the dark for 15 min
18. Add 2 ml VersaLyse
19. Vortex for 3 seconds
20. Incubate at room temperature in the dark for 15 min
21. Pellet cells by centrifugation at 200 g for 5 min
22. Aspirate supernatant using a vacuum pump
23. Vortex tube for 3 seconds
24. Add 3 ml DPBS
25. Pellet cells by centrifugation at 200 g for 5 min
26. Aspirate supernatant using vacuum a pump
27. Resuspend cells in 380 µl 0.1 % IOTest 3 Fixative Solution for data collection using a Navios flow cytometer

4. Sample preparation using DuraClone IM Tubes: Tregs

1. Collect peripheral blood samples directly into EDTA-vacutainers
2. Samples can be stored at 4 °C for a maximum of 4 h before processing
3. Mix the blood sample by repeatedly inverting the vacutainer
4. Transfer 50 µl blood into the DuraClone IM Treg Tube 1
5. Vortex for 8 seconds
6. Incubate at room temperature in the dark for 15 min
7. Add 3 ml DPBS
8. Pellet cells by centrifugation at 500 g for 5 min
9. Aspirate supernatant using vacuum pump
10. Vortex for 3 seconds
11. Add 50 µl heat-inactivated FCS
12. Mix by pipetting
13. Add 5 µl PerFix-nc Buffer 1
14. Vortex for 8 seconds
15. Incubate at room temperature in the dark for 15 min
16. Add 400 µl PerFix-nc Buffer 2
17. Vortex for 8 seconds
18. Transfer content of DuraClone IM Treg Tube 1 into DuraClone IM Treg Tube 2 by pipetting
19. Vortex Tube 2 for 8 seconds
20. Incubate at room temperature in the dark for 60 min
21. Add 3 ml DPBS
22. Incubate at room temperature in the dark for 5 min

23. Pellet cells by centrifugation at 500 g for 5 min

24. Aspirate supernatant using a vacuum pump

25. Vortex for 3 seconds

26. Add 3 ml 1X PerFix-nc Buffer 3

27. Pellet cells by centrifugation at 500 g for 5 min

28. Aspirate supernatant using a vacuum pump

29. Resuspend cells in 380 µl 1X PerFix-nc Buffer 3 for data collection using a Navios flow cytometer

Analysis of data obtained using DuraClone IM Tubes

Template manual gating strategies for extracting leucocyte subset frequencies from flow cytometry data using Kaluza software are given in Figure 1 to 7. Analyses are ideally performed by experienced operators blinded to clinical outcomes.

Fig. 1 DuraClone IM Phenotyping Basic Tube

Fig. 2 DuraClone IM T cell subsets Tube

Fig. 3 DuraClone IM TCRs Tube

Fig. 4 DuraClone IM Granulocytes Cells Tube

Fig. 5 DuraClone IM Dendritic Cells Tube

Fig. 6 DuraClone IM B cells Tube

Fig. 7 DuraClone IM Treg Tube

Troubleshooting

Time Taken

Anticipated Results

References

1. Hutchinson, JA. Predicting Early Viral Control under Direct-Acting Antiviral Therapy for Chronic Hepatitis C Virus Using Pretreatment Immunological Markers. Front Immunol. 7;9:146 (2018)

2. Kverneland, AH. Age and gender leucocytes variances and references values generated using the standardized ONE-Study protocol. Cytometry A. 89(6):543-64 (2016)

3. Streitz, M. et al. Standardization of whole blood immune phenotype monitoring for clinical trials: panels and methods from the ONE study. Transplant Res. 25;2(1):17 (2013)

Acknowledgements

Figures

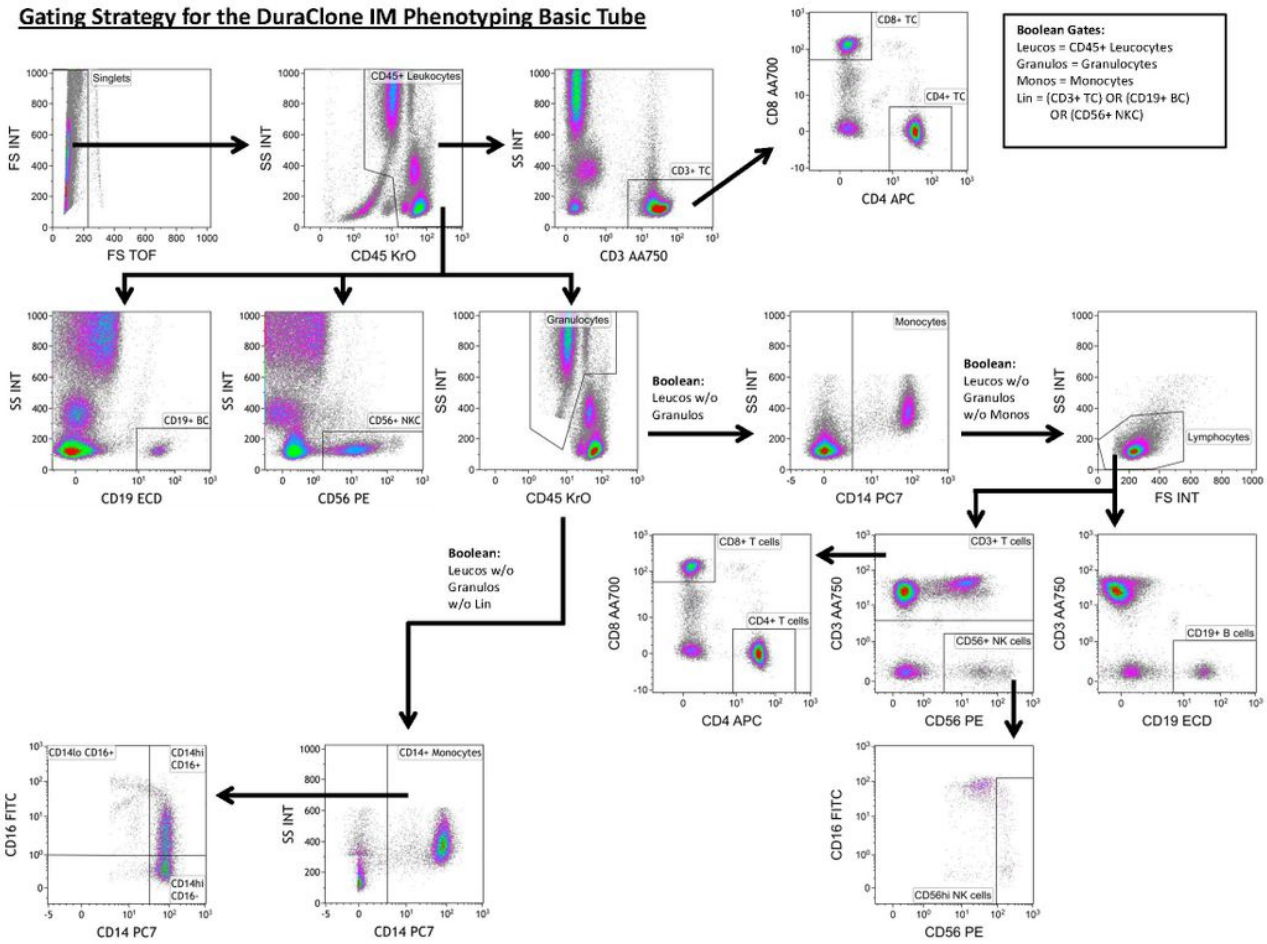

Figure 1

DuraClone IM Phenotyping Basic Tube

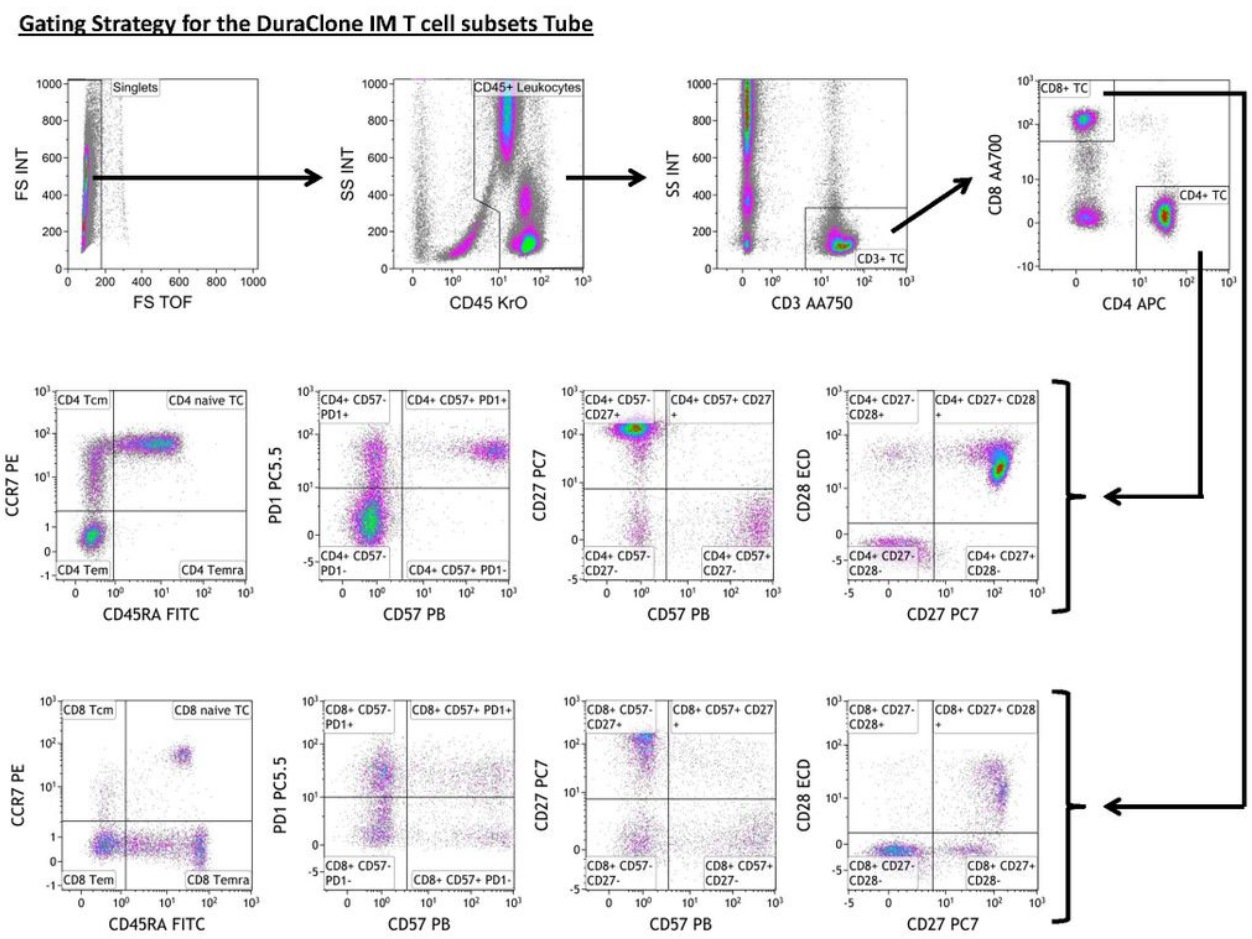

Figure 2

DuraClone IM T cell subsets Tube

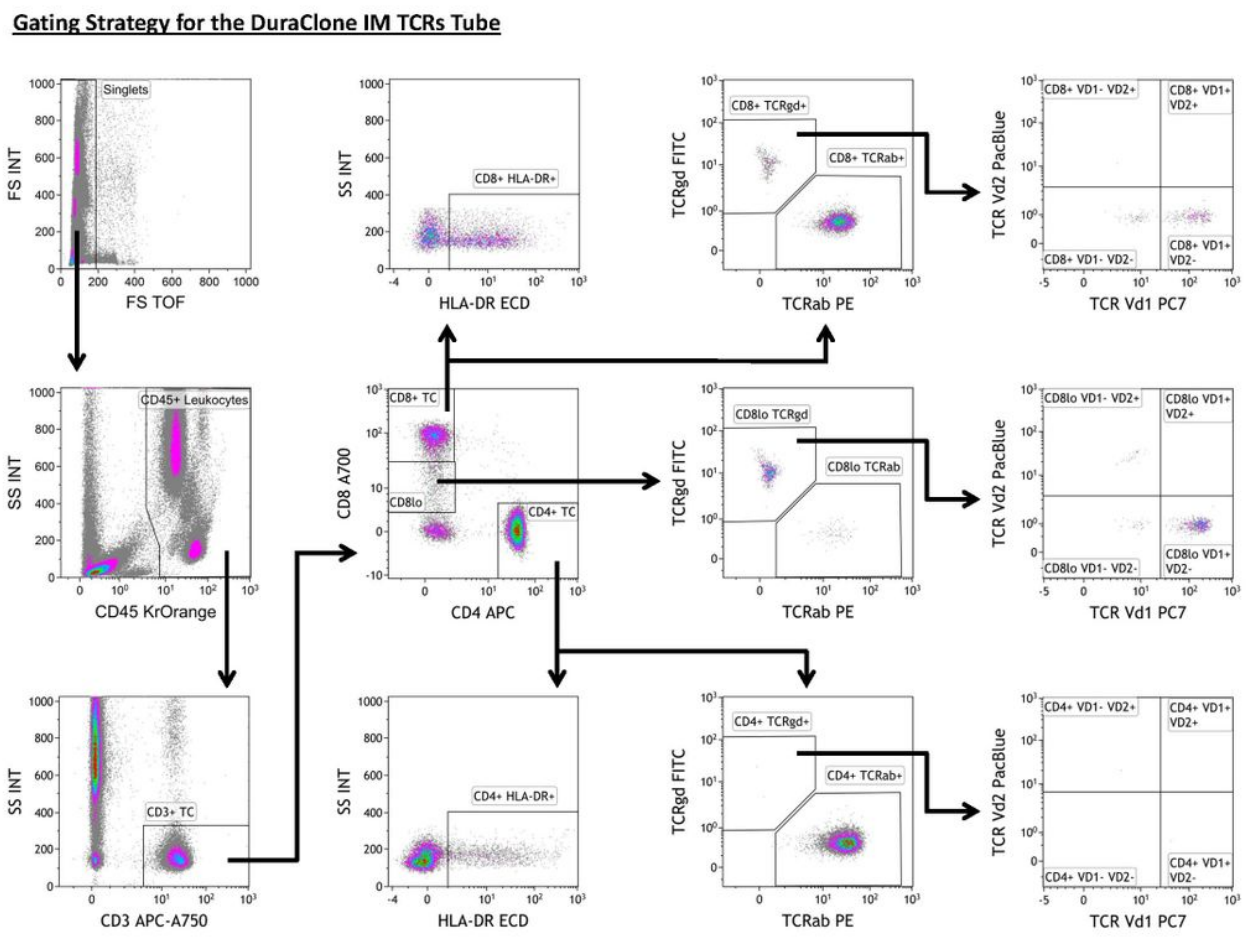

Figure 3

DuraClone IM TCRs Tube

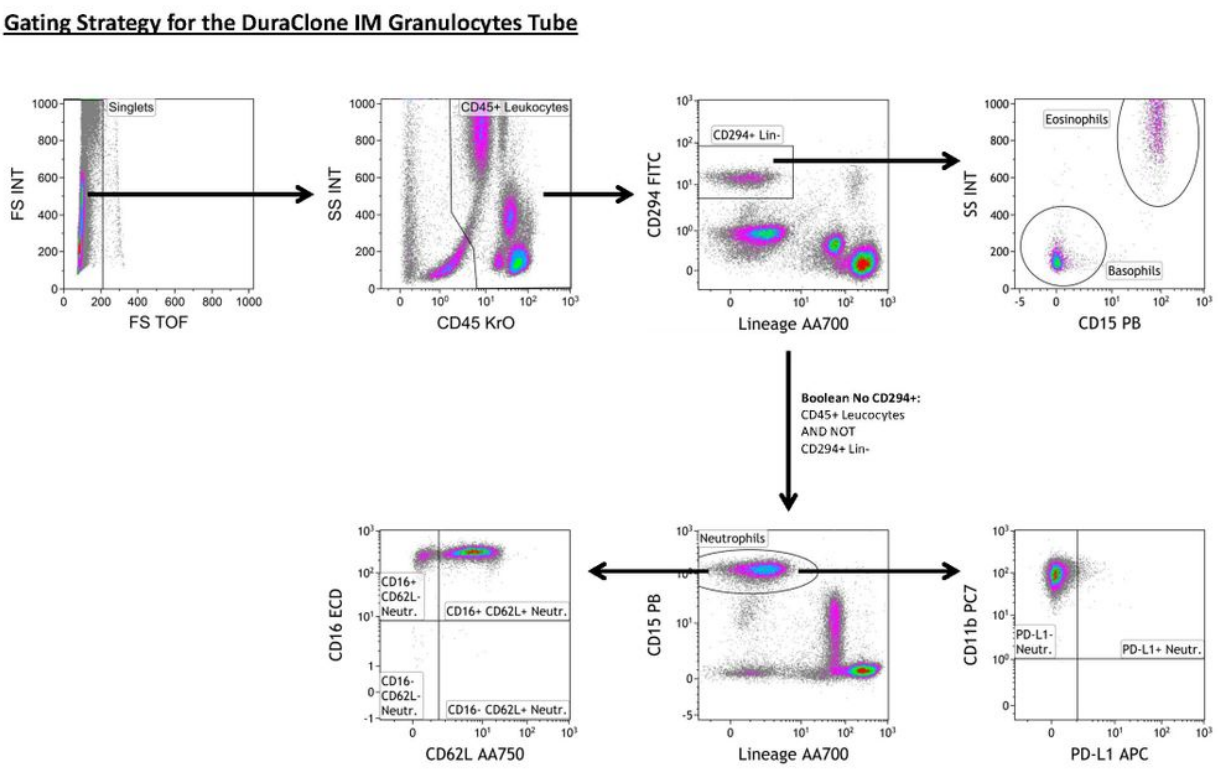

Figure 4

DuraClone IM Granulocytes Cells Tube

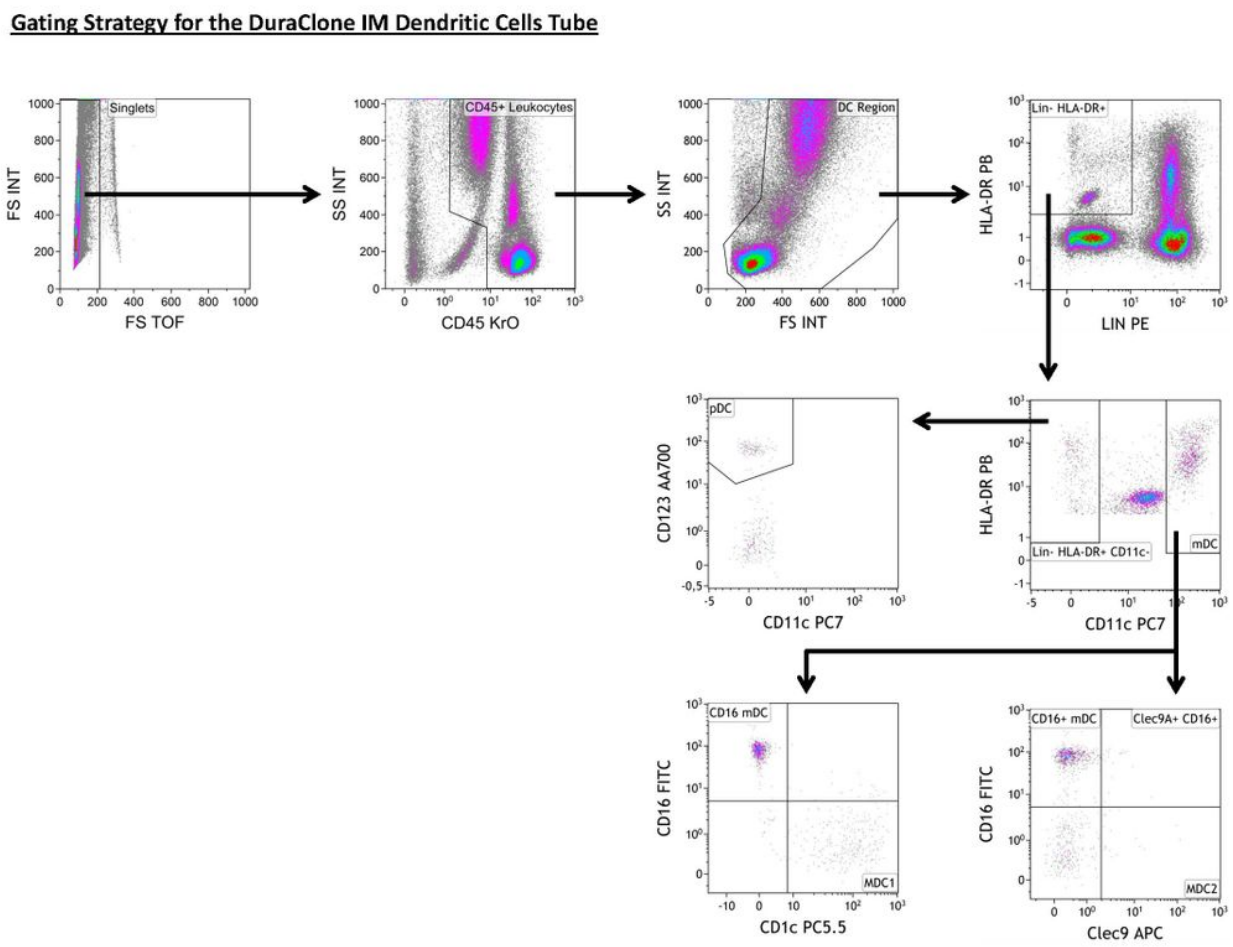

Figure 5

DuraClone IM Dendritic Cells Tube

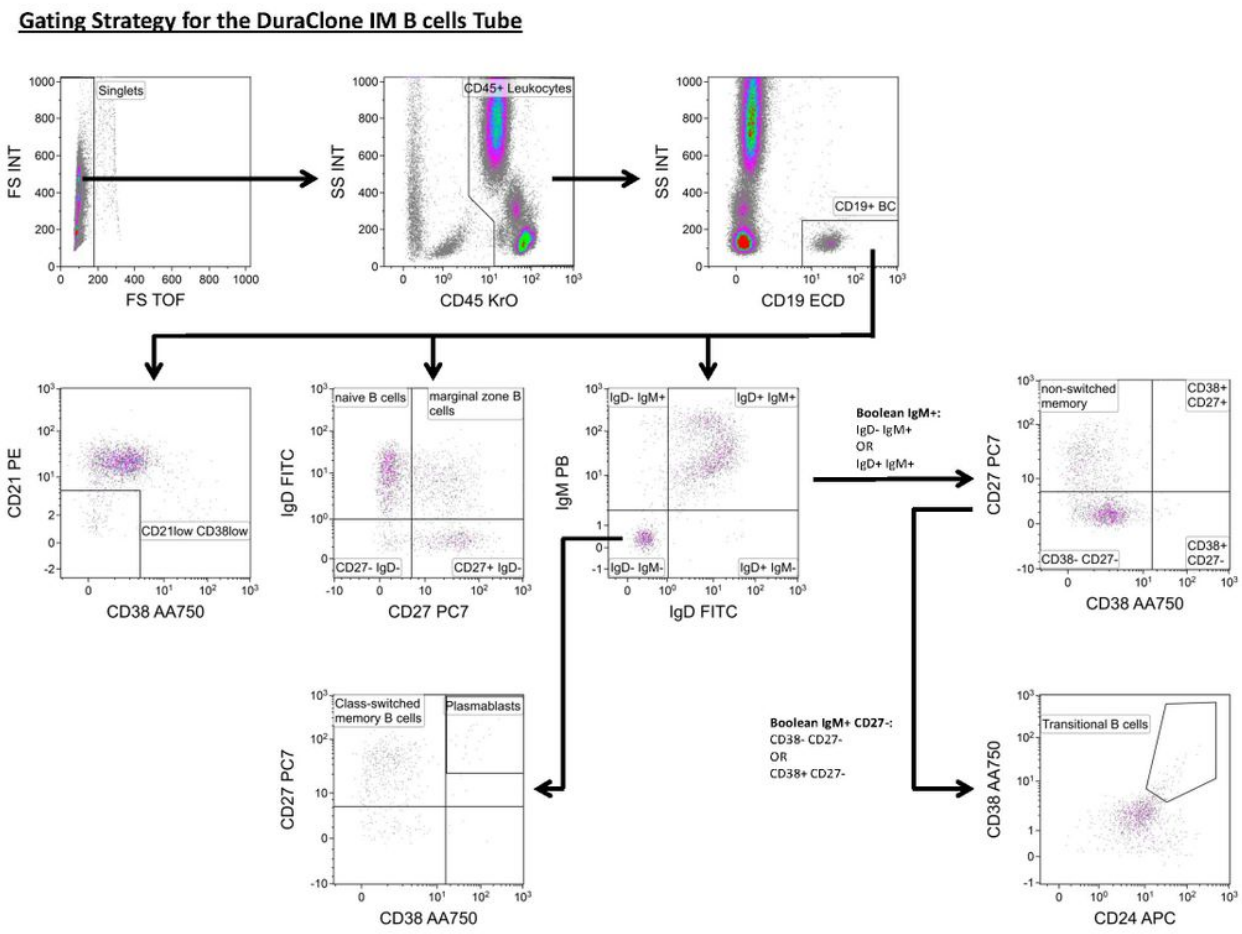

Figure 6

DuraClone IM B cells Tube

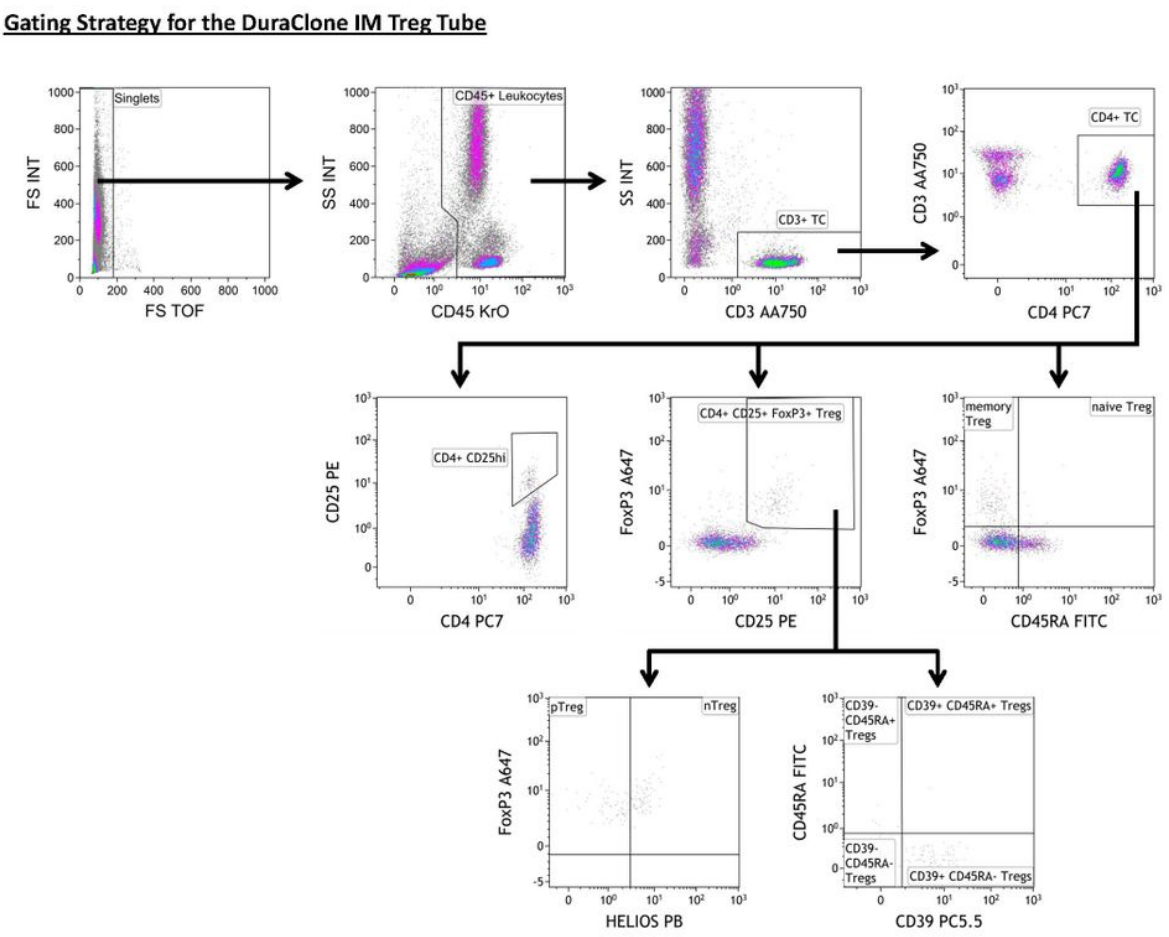

Figure 7

DuraClone IM Treg Tube