Mouse spleen tissue freezing with Bambanker™, a comparative study

Product	Bambanker™ (BB0#)
Distributor	NIPPON Genetics EUROPE GmbH
Manufacturer	GC Lymphotec Inc.

The following data is courtesy of a customer from Kyoto University.

Purpose

Investigate the effectiveness of Bambanker™ in tissue freezing and compare the results with those of competing products.

Summary

Slow cryopreservation reagents have been developed primarily for freezing isolated cells, with limited research into their efficacy for freezing tissues.
In this study, mouse spleens were frozen at -80°C for 10 days using commercially available slow cryopreservation reagents and the thawed cells were analysed using a flow cytometer.
Bambanker™ proved to be the optimal choice, demonstrating superior cell viability and integrity.

Materials

- Mouse spleens (BALB/c 8-10 weeks old)
- Bambanker™
- CELLBANKER2
- STEM-CELLBANKER GMP grade
- RPMI 1640 medium (Gibco)
- 10% FBS (CosmoBio)
- 1% penicillin-streptomycin (Gibco)
- gentleMACS™ Octo Dissociator (Miltenyi Biotec)
- ACK lysis buffer (0.15 M NH4Cl, 0.1 mM EDTA·2Na, 1 mM KHCO3, pH7.2-7.4)
- 1x PBS
- TC20 cell counter (Bio-Rad)

Experimental procedure

Evaluating cell viability

1. Survival rate assessed with a cell counter

Procedure

1. The spleens of three mice were collected.
2. One spleen was cut into four equal pieces, immersed in 1 mL of each of the three cryopreservation media (Bambanker™, CELLBANKER2 and STEM-CELLBANKER GMP grade) and stored at -80°C for 10 days.
3. After thawing in a water bath at 37°C, the spleen samples were immersed in RPMI 1640 containing approximately 10 mL of 10% FBS and 1% penicillin-streptomycin.
4. The samples were centrifuged and the supernatant was discarded.
5. Spleens were dissociated using Miltenyi Biotec's gentleMACS™ Octo Dissociator and cells were then isolated.
6. RBCs were haemolysed in ACK lysis buffer and resuspended in PBS.
7. Cell counts and viability were determined using a TC20 cell counter (Bio-Rad).

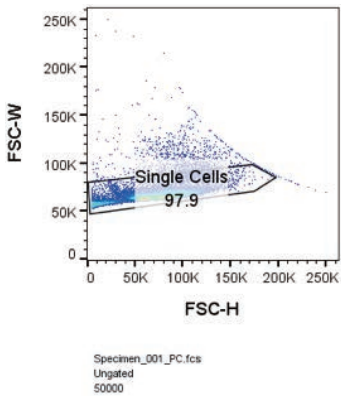
2. Cell viability assessed with a flow cytometer

Procedure

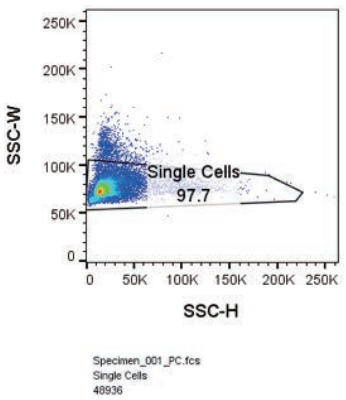
1. 10^6 cells were aliquoted from each cell suspension.
2. Dead cell staining was performed with eBioscience™ Fixable Viability Dye eFluor™ 506.
3. The samples were incubated with Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™).
4. Cell surface markers were stained with APC anti-mouse CD45, Pacific Blue anti-mouse CD4 and FITC anti-mouse CD19.
5. Naive mouse spleen cells were used as a control.
6. The results were analyzed using a BD FACSCanto II flow cytometer (Becton Dickinson).

Data analysis workflow

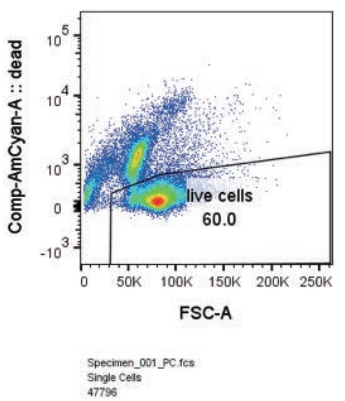
a) Doublet removal in FSC



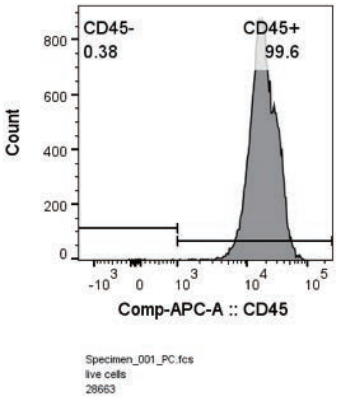
b) Doublet removal in SSC



c) Dead cells and living cells: separate and gate live cells



d) Lymphocyte marker CD45
Gate positive cells



e) Helper T-cell markers, CD4 and B cell markers Expanded with CD19
Cell status assessment

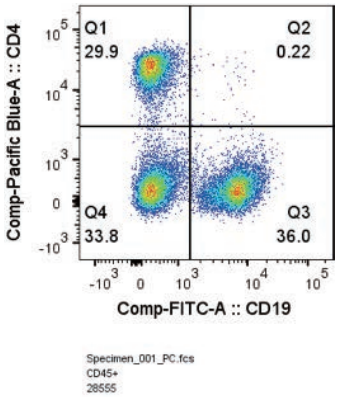


Figure 1: Data analysis workflow of flow cytometry.

Results

Cell survival rate

I. Cell counter

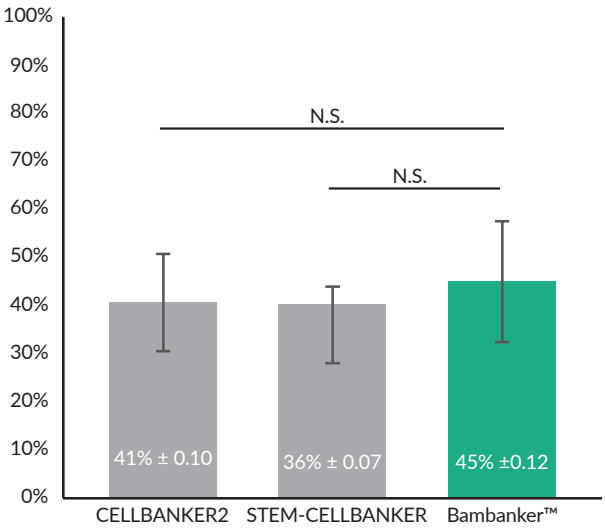


Figure 2: Number of living cells assessed by cell counter after cryopreservation of mouse spleen cells in 3 different cryopreservation media.
N.S. - Not Significant

II. Flow cytometry

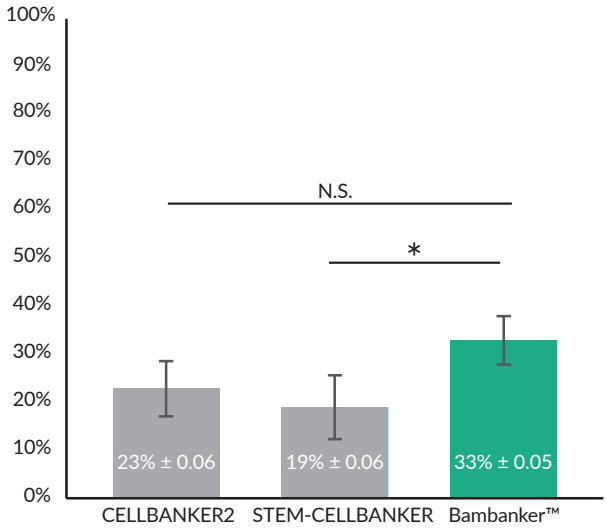


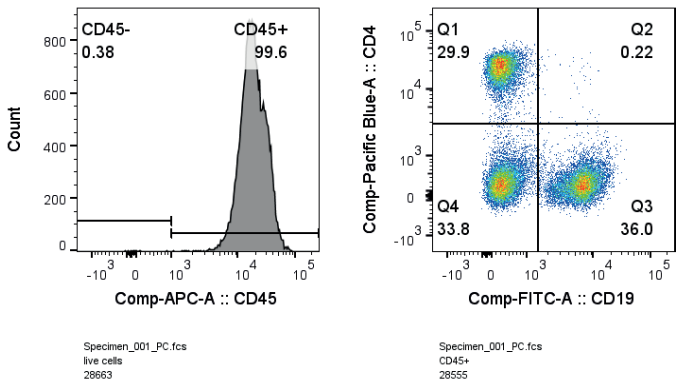
Figure 3: Number of living cells assessed by flow cytometer after cryopreservation of mouse spleen cells in 3 different cryopreservation media.
** p < 0.05 (Student t-test; n= 3)*
N.S. - Not Significant

Although no clear differences were found, both study methods showed the highest survival rates (45% ± 0.12 and 33% ± 0.05) for Bambanker™.

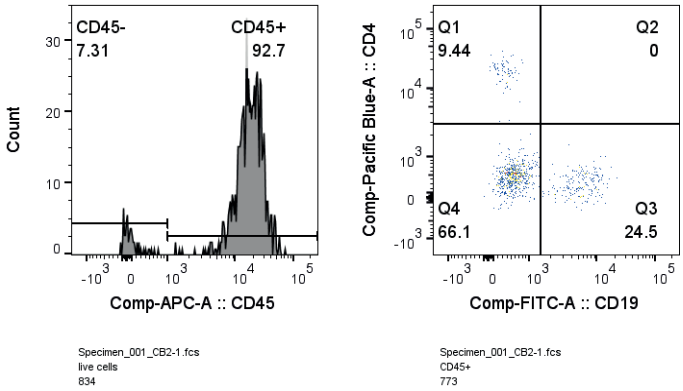
Cell viability

I. FACS

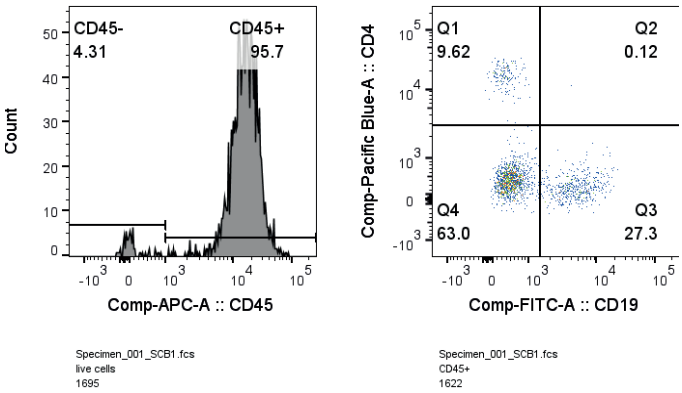
Control



CELLBANKER2



STEM-CELLBANKER



Bambanker™

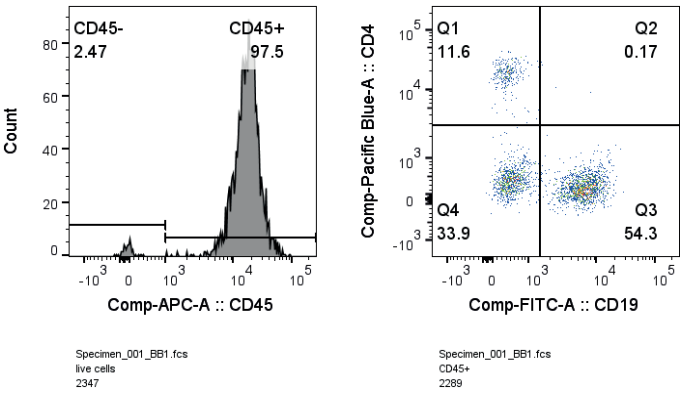


Figure 4: Identification of cells subsets from a mouse spleen after being cryopreserved for 10 days with three different cryopreservation media: CELLBANKER2, STEM-CELLBANKER, and Bambanker™.

Cell surface markers were labeled with with APC anti-mouse CD45, Pacific Blue anti-mouse CD4 and FITC anti-mouse CD19.

Cells cryopreserved in Bambanker™ had a higher proportion of cells in Q1 (CD4 positive) and Q3 (CD19 positive).

Conclusion

In contrast to its competitors (CELLBANKER2 and STEM-CELLBANKER GMP grade), Bambanker™ demonstrated notably higher survival rates and cell viability when cryopreserving mouse tissue. This ensured the effective preservation of T and B cells. However, it is important to note that the cryopreservation of different types of tissue needs to be further investigated.

Customer comment

Performing cell separation immediately after tissue collection often leads to increased manipulation. To mitigate this, freezing the tissue immediately after collection serves as a convenient stopping point for the work. Given the satisfactory results obtained with Bambanker™ this time, we aim to use it for this purpose in the future.

Cell survival rate experiment

In the approach involving immediate processing of harvested tissues (cell dissociation and lysation) and subsequent cryopreservation with CELLBANKER2, cell survival after thawing was consistently within the range of 85-95%. However, in our present experiment, as outlined here, we have observed diminished cell survival rates. We hypothesize that this discrepancy could be attributed to two factors: i) assessing cell viability post-thawing and tissue grinding, and ii) insufficient exploration of the optimal thawing method for the tissue.

Cell viability experiment

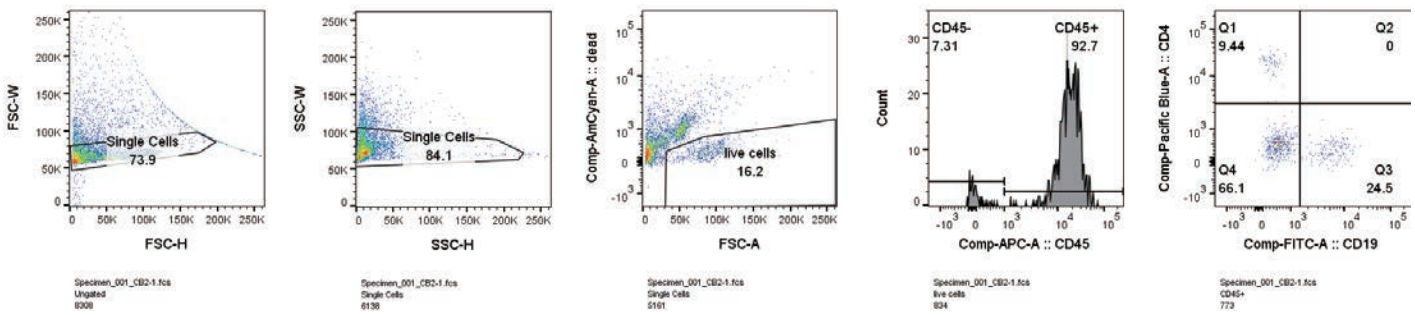
In this experiment, both CELLBANKER2 and STEM-CELLBANKER GMP grade produced comparable results, indicating inadequate staining for CD4 and CD19 along with a tendency toward low survival rates. We believe that this aspect could be enhanced by refining the thawing method and subsequent treatment. However, we are currently unable to explore this further. On a positive note, the cell survival rate using the Bambanker™ appears promising, with a minimal occurrence of doublets. However, given the continued challenge of inadequate CD4 staining, there remains potential for further exploration into the efficacy of Bambanker™.

Supplementary data

CELLBANKER2

n = 1

a) *b)* *c)* *d)* *e)*



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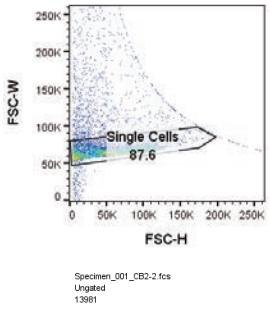

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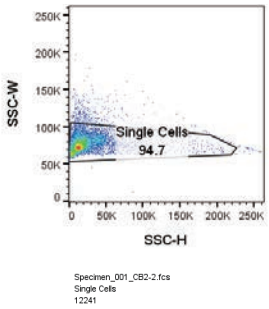

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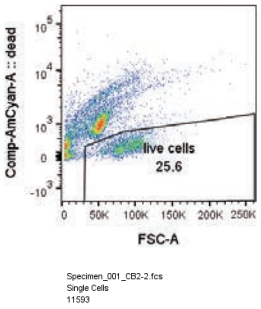

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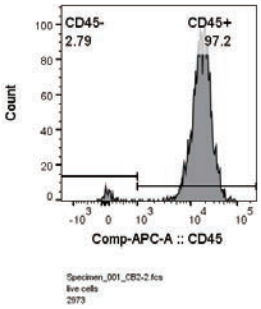

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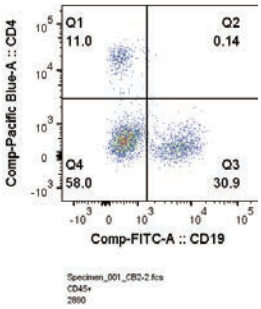

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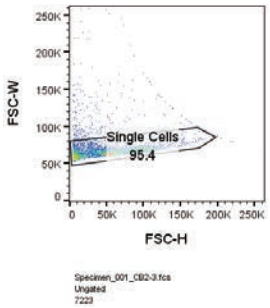

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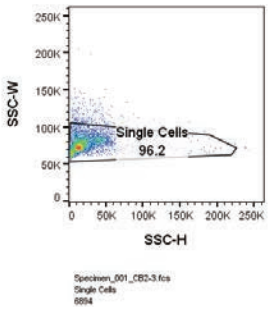

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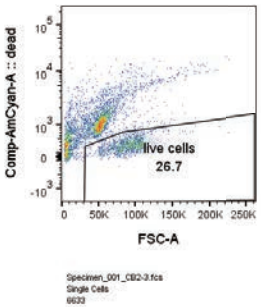

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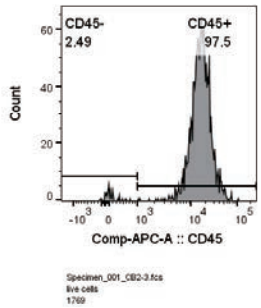

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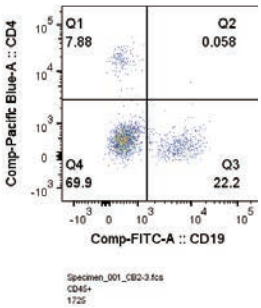

STEM-CELLBANKER GMP grade

n = 1

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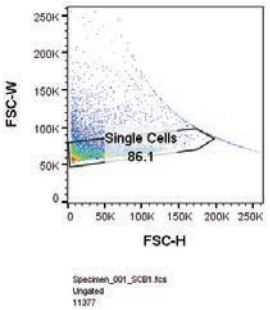

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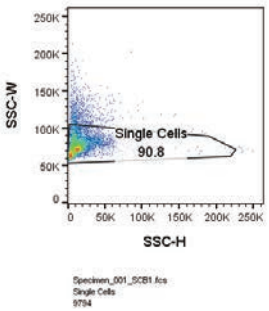

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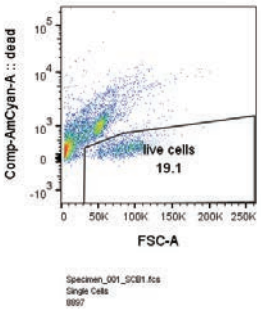

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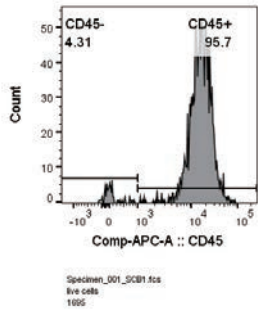

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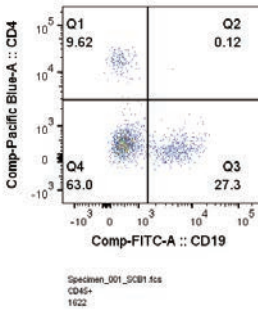

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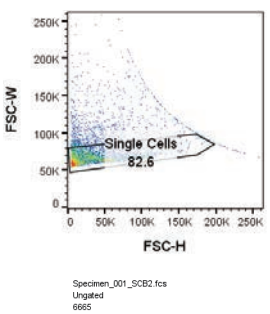

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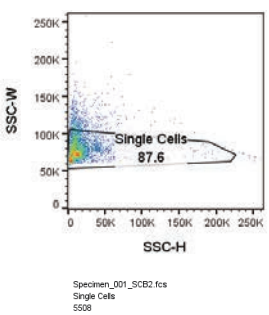

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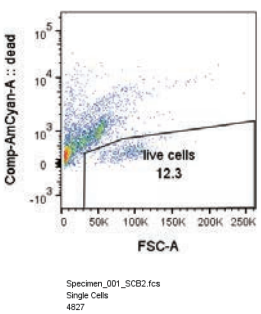

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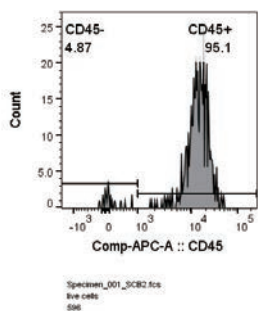

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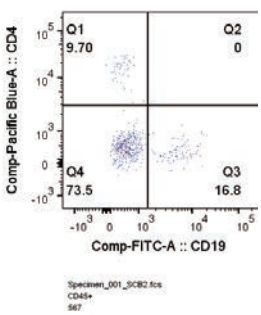

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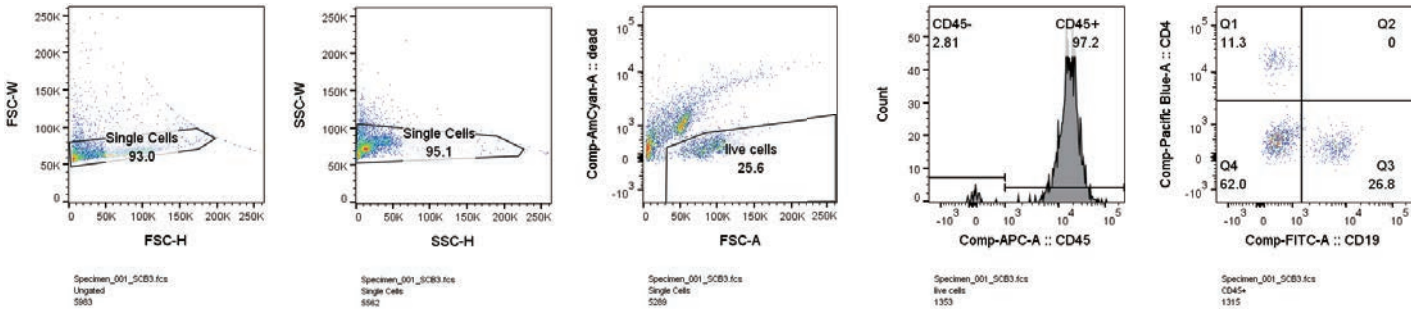

Bambanker™

n = 1

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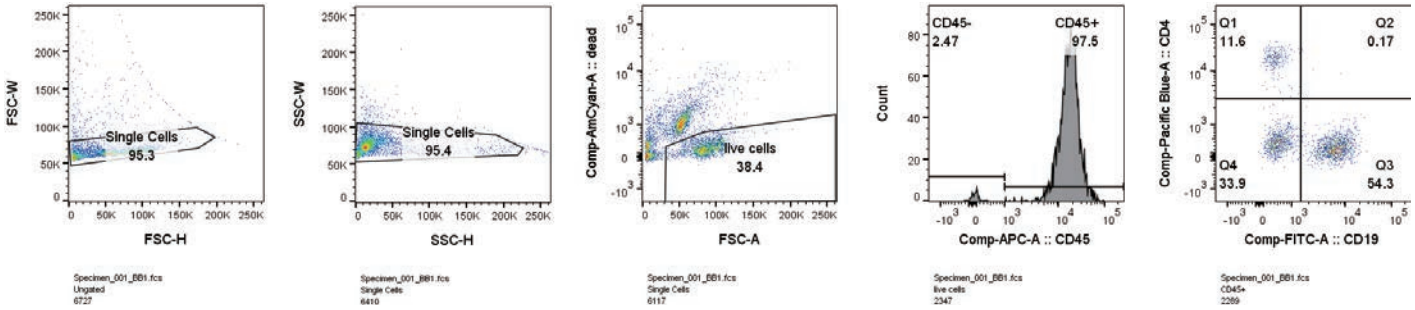

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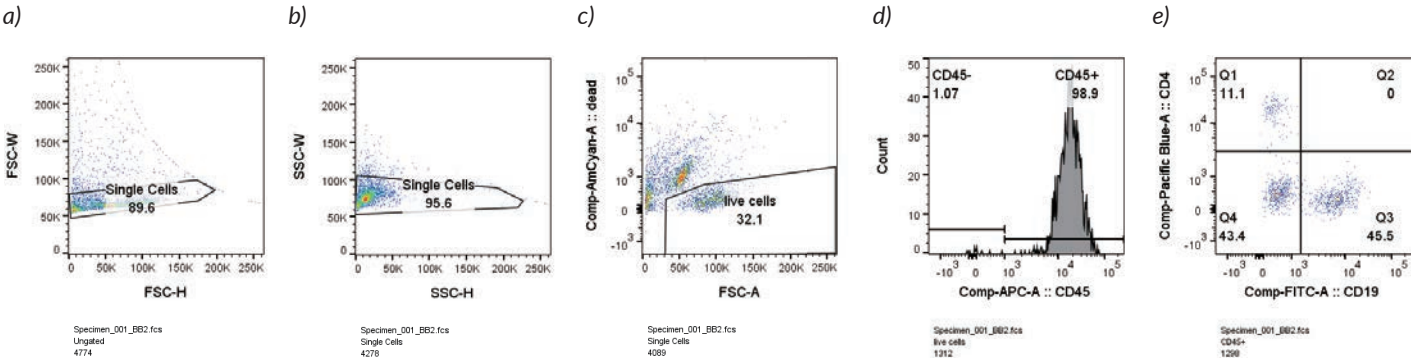

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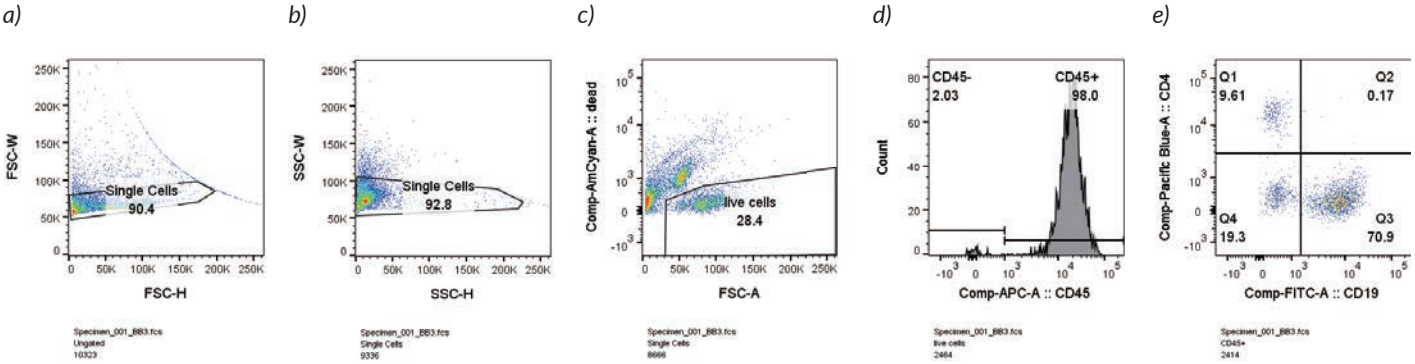

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