


# Best practices in optimizing sample processing for improved flow cytometry



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## Biosketch

Chris Spring has been working as a flow cytomestrist in the cell biology field for over 15 years, primarily working on analyzing the role of platelets in immunological and cardiovascular diseases. Most of his scientific career has been spent in laboratories in St Michael's Hospital, as well as McMaster University and the Australian Centre for Blood Diseases. He is currently co-President of the Canadian Microscopy and Cytometry Association and has been involved with them since 2012.

Chris was featured in our LabRoots webinar on November 30, 2016. For more information, please go to our website:

### gentleMACS™ Octo Dissociator with Heaters



"The gentleMACS™ Octo Dissociator with Heaters has allowed us a better methodology in terms of tissue dissociation"

### autoMACS® Pro Separator



"Our experiments would not have been possible without the pre-enrichment steps on the autoMACS® Pro Separator"

### MACSQuant® Flow Cytometer



"The QC is incredibly easy, you just barcode scan the bottle, put a drop in a tube, anyone can do it"

# Webinar highlights

## Experimental Setup

Mouse splenocytes were first isolated by manual dissociation, after which CD4$^+$ T cells were enriched on the autoMACS® Pro Separator. Following pre-enrichment, the CD25$^+$ population was isolated for downstream analysis of viability and purity as well as an adoptive transfer into mice to study an immunological model of disease (Figure 1).

### Rare-cell enrichment using the autoMACS® Pro Separator

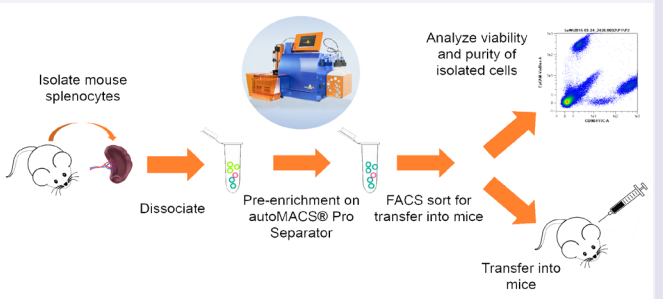



**Figure 1.** Schematic illustrating the isolation of rare CD25$^+$ cells from mouse spleen.

## Results

Prior to pre-enrichement on the autoMACS® Pro Separator, the CD25$^+$ population was only approximately 2% of the total population, which made it very difficult to isolate enough cells for adoptive transfer into mice (Figure 2).

### Flow analysis data before enrichment

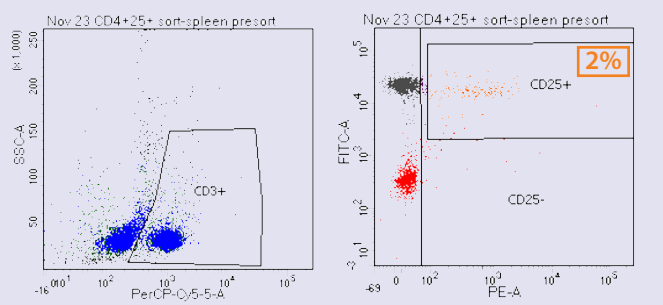



**Figure 2.** Flow cytometry analysis of CD3$^+$ positive cells isolated from mouse spleen. Splenocytes (blue), CD4$^+$ T cells (grey) and CD25$^+$ T cells (orange) are displayed. The CD3$^+$, CD4$^-$ population is represented in red.

Following pre-enrichment for CD4$^+$ T cells on the autoMACS® Pro Separator, the CD25$^+$ population increased to around 8% (Figure 3).

### Flow analysis data after enrichment

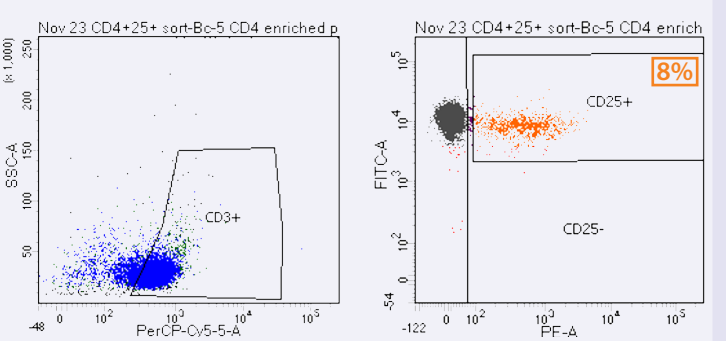



**Figure 3.** Flow cytometry analysis of CD3$^+$ positive cells isolated from mouse spleen. Splenocytes (blue), CD4$^+$ T cells (grey) and CD25$^+$ T cells (orange) are displayed. The CD3$^+$, CD4$^-$ population is represented in red.

Following isolation of the CD25$^+$ population on the autoMACS® Pro Separator, these cells were transferred to a flow sorter, isolated by flow analysis, analyzed for purity and viability, and directly transferred into mice (Figure 4).

### Analysis of flow sorted population before transfer into mice

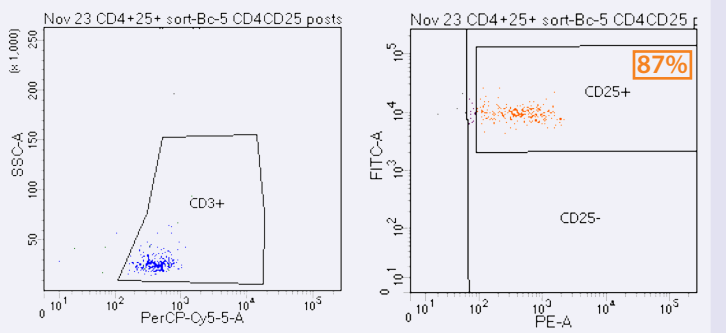



**Figure 4.** Flow sorting analysis of CD3$^+$ positive cells isolated from mouse spleen. Splenocytes (blue), CD4$^+$ T cells (grey) and CD25$^+$ T cells (orange) are displayed.

## Summary

The pre-enrichment step on the autoMACS® Pro Separator allowed for isolation of enough cells to perform the adoptive mouse experiments. Without this pre-enrichment step, it was not feasible to carry out a cell sorting experiment and expect to isolate enough cells for downstream analysis.


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