

# A Simple, Column-Free Method for Positive Selection of Functional Dendritic Cells from Normal, Knockout and Transgenic Mouse Strains

Maureen Fairhurst<sup>1</sup>, Steve Woodside<sup>1</sup>, Terry E. Thomas<sup>1</sup>, Amy Tien<sup>2</sup>, Leanne Berrington<sup>2</sup>, and Cheryl D. Helgason<sup>2</sup>

<sup>1</sup>StemCell Technologies Inc, Vancouver, BC, Canada; <sup>2</sup>Cancer Endocrinology, British Columbia Cancer Agency, Vancouver, BC, Canada

## Introduction

Dendritic cells (DC) play a pivotal role in antigen presentation during the generation of primary T cell responses. They may be isolated from primary tissues or derived *in vitro* by various strategies. We have developed an immunomagnetic positive cell selection technique called EasySep™ that does not rely on columns and utilizes nanoparticles compatible with flow cytometry. This method has been successfully used to purify CD11c<sup>+</sup> DC from unfractionated spleen cell preparations from normal (WT), SHIP<sup>-/-</sup> knockout and LPB-Tag transgenic (Tg) mice. SHIP is thought to play a role in regulating DC development and function, while the Tg mice are used as a model for human prostate cancer. Using the EasySep™ method, CD11c<sup>+</sup> DC selected from WT, Tg and young (<2 months) SHIP<sup>-/-</sup> mice upregulated DC associated antigens (CD40, CD80, CD86, I-A) upon stimulation with LPS or GM-CSF and IL4, and effectively induced allogeneic T cell proliferation. DC from older (>4 months) SHIP<sup>-/-</sup> mice showed an elevated frequency of CD11c<sup>+</sup> DC, impaired upregulation of CD40 and I-A, and impaired ability to induce proliferation, revealing age related changes in these mice. These results suggest that fully functional DC can be successfully isolated by EasySep™. This simple and rapid technique will facilitate studies of CD11c<sup>+</sup> DC function from normal, knockout and transgenic mice at the cellular and molecular level.

## Methods

Figure 1.

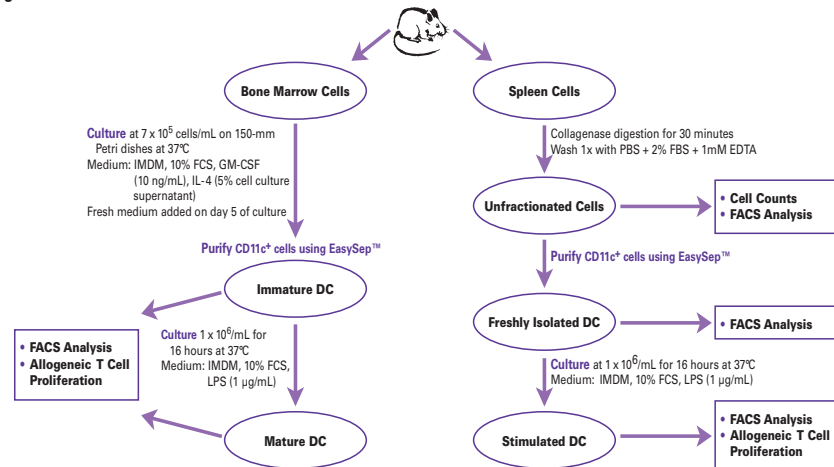
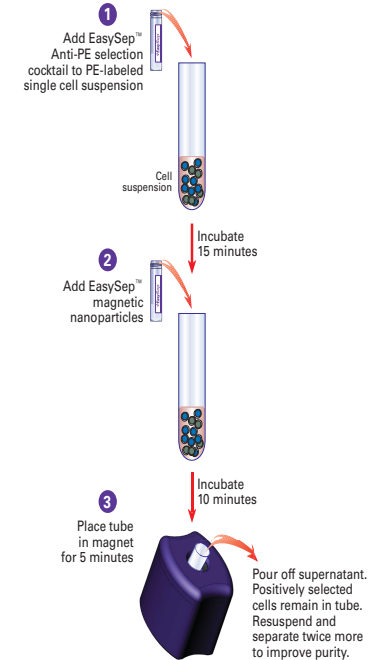


Figure 2. EasySep™ procedure for cell selection



**Optimizing Purity.** For samples with low starting numbers of target cells, less than 10%, one or two additional wash/separation rounds will improve purity. Recovery may decrease with each extra round of wash/separation.

**Detection of Enriched Cells.** The positively selected cells have already been labelled with the PE-conjugated antibody and purity can be assessed directly by flow cytometry.

## Results

Table 1. % Purity and % recovery of splenic DC or cultured bone marrow derived DC from normal (WT), transgenic (12T-TG) or SHIP<sup>-/-</sup> mouse strains after EasySep™ positive selection

Strain	Spleen $\bar{x} \pm 1$ SD, n=4		Bone Marrow n=2	
	% Purity	% Recovery <sup>1</sup>	% Purity	% Recovery <sup>1</sup>
CD1	12T-WT	97 ± 1	12 ± 3	ND
	12T-TG	97 ± 3	25 ± 9	ND
C57x129	SHIP WT	91 ± 2	20 ± 4	97, 97
	SHIP <sup>-/-</sup>	92 ± 6	30 ± 11	95, 96

<sup>1</sup> Number of cells expressing CD11c in the enriched fraction divided by the number of CD11c in the start fraction.

Figure 3. Expression of DC associated antigens on enriched splenic DC after stimulation with LPS

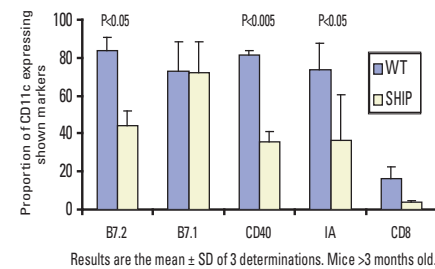
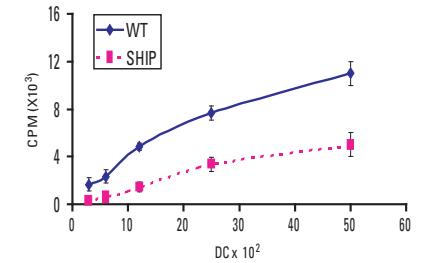


Figure 4. Proliferation of allogeneic T cells in response to splenic DC (WT or SHIP<sup>-/-</sup>) selected by EasySep™ and stimulated with LPS



CD11c<sup>+</sup> DC were isolated by EasySep™ and added to 10<sup>5</sup> allogeneic SpinSep™ purified T cells. DC alone or T cells without DC were also plated as negative controls. Cells were cultured in 96-well U-bottom plates for 3 days and pulsed with <sup>3</sup>H Thymidine during the final 24 hours. Results are the mean ± SD of triplicate determinations from a representative experiment (n=3).

## Conclusions

- Expression of DC associated antigens on splenic DC is altered in SHIP deficient mice.
- Older SHIP<sup>-/-</sup> mice show impaired ability to stimulate T cell proliferation.
- DC can be enriched from cultured murine bone marrow and spleen using EasySep™.
- No columns required.
- High cell purity - utilizes the specificity of antibody-mediated selection.
- Freshly selected CD11c<sup>+</sup> cells do not show upregulation of co-stimulatory molecules (data not shown). Upon stimulation with LPS, cells develop typical phenotype of mature, activated DC.
- EasySep™ selected DC can function as APCs in a Mixed Lymphocyte Reaction.