



# TECHNICAL NOTE

## ROBOSEP™ APPLICATIONS

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### Introduction

Cell separation is a powerful technique for isolating cells of interest or depleting unwanted cells from a heterogeneous cell population. RoboSep™, the fully automated cell separator based on the column-free EasySep™ technology, is the first instrument to allow true walk-away automation of cell separation. Cells from virtually any source or any species can be isolated with RoboSep™ using negative (depletion of unwanted cells) or positive (selection of desired cells) selection protocols. As many as four samples can be simultaneously labeled and separated using disposable tips that ensure no cross-contamination between samples. In addition, RoboSep™ is sized to fit within most standard bio-containment hoods to ensure sterile operation. Following separation with RoboSep™, cells are immediately available for flow cytometry, culture or other downstream applications.

### Cell Sources

RoboSep™ can isolate cells from virtually any source. To date, it is possible to separate cells of human, mouse, rat, primate and other origins. The most commonly used starting cell preparations are of hematopoietic origin from peripheral blood, cord blood, bone marrow or spleen, but cells from other tissues (e.g. mammary, bone and adipose tissue) are also suitable, provided single cell suspensions can be generated.

### Separation Methods

Both positive and negative selection strategies can be used to isolate cells with RoboSep™. In positive selection, the cells of interest are labeled with a specific cell surface antigen and then crosslinked to magnetic particles using bi-specific antibody complexes to create a tetrameric antibody complex (TAC). The TAC comprise two mouse IgG, monoclonal antibodies; one mouse antibody that recognizes the specific cell surface antigen, and another that recognizes the dextran-coated magnetic particles. In negative selection, the

unwanted cells are targeted for depletion using multiple TACs, which target all cell types except the cell of interest, and crosslink them to magnetic particles. The labeled, unwanted cells remain inside the tube when placed in the magnet, while the desired, unlabeled cells are transferred to a new tube. RoboSep™ can process up to 4 different samples simultaneously.

### Applications

RoboSep™, the first fully automated cell separator with true walk-away technology, is a unique and innovative instrument, providing researchers with an efficient and easy-to-use option for the enrichment of cell populations for various downstream applications. The device can significantly reduce the time required by staff for routine processing procedures from multiple samples.

### Flow Cytometry Core Centers

Fluorescence activated cell sorting (FACS) is commonly used to isolate pure populations of cells. However, for rare cell types this can be a time-consuming and impractical method that may result in low yields and viability. To reduce the time required for cell sorting, pre-enrichment of cells can be performed using RoboSep™.



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## TECHNICAL NOTE

There are several kits available for the pre-enrichment of different cell populations, including lineage depletion kits for enrichment of hematopoietic progenitors, granulocyte depletion (or enrichment), and Glycophorin A depletion (or enrichment). Using RoboSep™ to pre-enrich cell populations will reduce the time required on the flow cytometer when sorting for rare populations, thus increasing the number of samples that can be sorted at flow cytometry core centers.



### HLA Labs

The HLA (human leukocyte antigen) complex, the human counterpart of the major histocompatibility complex in mice, encodes genes essential for immune function and plays a critical role in transplant and transfusion medicine. HLA typing is critical for transplantation, to ensure that the donor tissue HLA type is compatible with that of the recipient, thus reducing the risk of transplant rejection. Isolating cells for HLA analyses can be performed with RoboSep™. Populations of total lymphocytes, B or T cells can be enriched with RoboSep™ using either positive or negative selection protocols. Both positive and negative selection can be performed on peripheral blood mononuclear cell (PBMC) samples or directly on whole blood. Cells isolated using RoboSep™ are immediately available for downstream HLA applications including crossmatch or chimerism analysis.

### Chimerism Analysis

Following allogeneic hematopoietic cell transplantation, patients are monitored for disease relapse, graft rejection, graft vs. host disease and other unfavorable outcomes. Chimerism analysis is performed on different cell populations isolated from patient blood in order to detect the presence of donor-derived cells. As the PCR method used for chimerism analysis is highly sensitive, the different cell populations used must be extremely pure. RoboSep™ is an ideal tool for the enrichment of lineage-specific cell populations required for chimerism analysis. Cells can be enriched with high purity and recovery from small volumes (2 - 3 mL) of blood.

Numerous selection strategies are available for RoboSep™ including positive selection protocols for T cells (CD3), B cells (CD19), myeloid cells (CD14, CD15, and CD33), NK cells (CD56), and progenitors (CD34). Additionally, RoboSep™ has the unique capacity to perform sequential separations, should the user need to isolate multiple populations from a single patient sample.

### Molecular Diagnostics

Cells that have been isolated using RoboSep™ can also be used for subsequent molecular diagnostics. Polycythemia vera (PV) is a myeloproliferative disease (MPD) characterized by increased red blood cell production. A recurrent clonal mutation causing a valine to phenylalanine substitution at position 617 (V617F) in JAK2 was recently identified as the underlying cause of PV and other MPDs such as essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). A PCR assay is used to screen for the JAK2-V617F mutation in granulocytes as a first intention diagnostic work-up for PV. To facilitate this, enrichment of granulocytes from patient

samples can be performed on RoboSep™ using a positive selection protocol for the CD66b antigen. The highly enriched granulocyte population can then be used in a PCR assay to detect the presence of the JAK2-V617F mutation.

## HIV

The human immunodeficiency virus (HIV) can lead to immune system failure and acquired immunodeficiency syndrome (AIDS). RoboSep™ is a useful tool for HIV research labs that need to isolate the immune system cells involved in the pathogenesis of HIV infection. HIV primarily infects cells of the human immune system such as CD4<sup>+</sup> helper T cells, macrophages and dendritic cells. HIV infection leads to reduced levels of CD4<sup>+</sup> T cells by direct viral killing of infected cells, increased rates of apoptosis in infected cells or by CD8<sup>+</sup> cytotoxic lymphocyte-mediated killing of infected CD4<sup>+</sup> T cells. Protocols are available for enrichment of CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells by either positive or negative selection, and for pre-enrichment of dendritic cells. Use of RoboSep™ minimizes sample handling thus reducing the researcher's risk of exposure to potentially hazardous pathogens.

## Multiple Myeloma

Multiple myeloma (MM) is a form of hematological neoplasia characterized by excessive numbers of malignant plasma cells. Plasma cells are the antibody-secreting cells of the immune system that differentiate from B cells upon stimulation by CD4<sup>+</sup> lymphocytes. In an immune response, pathogens are taken up by B cells via receptor-mediated endocytosis, broken down to antigen peptides upon fusion with lysosomes, and then presented on the extracellular surface of MHC class II molecules. CD4<sup>+</sup> T helper lymphocytes will bind to the MHC II/antigen complex, causing B cell activation and differentiation into a plasma cell, which generates antibodies against the endocytosed pathogen. Plasma B cells originate in the bone marrow, and then travel to the spleen or lymph nodes to secrete antibodies. The CD138 antigen (Syndecan-1) is a universally accepted marker of normal and malignant plasma cells. For labs wishing to study plasma cells, a CD138 enrichment protocol is available for RoboSep™. The CD138 selected cells can be used to identify the tumor clone in peripheral blood, bone marrow or leukapheresis products by PCR analysis of the Ig heavy chain using allele-specific oligonucleotide primers. A highly pure CD138 population is crucial for the consensus PCR. Plasma CD138<sup>+</sup> cells are also used for the identification and

isolation of the specific tumor antigen idotype for designing idotype-based dendritic cell vaccination for MM. New dendritic cell-based immunization strategies include RNA transfection of dendritic cells. RoboSep™ offers a time-saving advantage to isolate CD138 cells for subsequent use in various downstream applications.

## Hematopoietic Stem and Progenitor Cells

A stem cell can be defined as a cell that has the ability to self-renew, giving rise to more stem cells, and the ability to differentiate, giving rise to clonal progeny. The hematopoietic stem cell (HSC) has the ability to self-renew and to differentiate giving rise to all cell types of the blood system. The HSC is responsible for developing, maintaining and regenerating the blood forming tissues throughout life and is thus the most important cell in hematopoietic cell transplants (HCT). Isolating HSCs is challenging as they represent a very small percentage of the hematopoietic system and no single unique marker exists that definitively identifies HSCs. However, it has been accepted that human HSCs express CD34 and are negative for lineage cell markers such as B cell, T cell and myeloid cell markers. Protocols for enrichment of stem and progenitor populations can be performed on RoboSep™ using a CD34 positive selection or lineage depletion strategy.

## Custom Protocols

To date, there are over 150 protocols specifically designed for RoboSep™ that enable automated selection of different cell populations. Importantly, RoboSep™ is equipped with a programmable interface such that custom protocols can be specifically designed for unique applications not currently available in the EasySep™/RoboSep™ product line. This unique feature allows for unlimited cell separation possibilities.



## RoboSep™ Equipment

### RoboSep™ The Fully Automated Cell Separator

**PRODUCT:** RoboSep™  
**CATALOG #:** 20000

#### SYSTEM IS SUPPLIED WITH:

- 4 “The Big Easy” EasySep™ Magnets
- 8 RoboSep™ Filter Tip Racks (1 box)
- 1 RoboSep™ Buffer (250 mL)
- RoboSep™ Carousel
- RoboSep™ Service Rack
- Hydraulic Fluid Bottle and Tubing
- User Manual
- One-Year Warranty

#### REQUIRED REAGENTS:

RoboSep™ Reagent Kit which includes:

- EasySep™ Selection Kit
- 8 RoboSep™ Filter Tip Racks (1 box)
- RoboSep™ Buffer (250 mL)

#### DESCRIPTION:

RoboSep™ is the first instrument to offer true walk-away automation of magnetic cell separation. Using column-free EasySep™ technology to isolate cells by either positive or negative selection, RoboSep™ can label and separate up to 4 samples at one time. The instrument is easily controlled via the integrated color touch-screen. The pre-optimized cell separation protocols are quickly selected using the intuitive graphical user interface. Once the cell samples and the appropriate reagents are loaded into the instrument, RoboSep™ performs all the steps necessary to magnetically label and separate the cells. The instrument handles the samples and reagents using disposable tips, eliminating cross-contamination. After completion of the cell separation cycle, the cells of interest are immediately available for any application. RoboSep™ fits in a standard bio-containment hood for sterile operation.

#### RECOMMENDED FOR:

Simultaneous separations of up to 4 samples with minimal hands-on time; sequential separations from the same sample.

#### CAPACITY:

- **Positive selection:** up to 4 different simultaneous separations; up to  $8 \times 10^9$  total cells (four samples of up to  $2 \times 10^9$  cells each).
- **Negative selection:** up to 4 different simultaneous separations; up to  $4 \times 10^9$  total cells (4 samples of up to  $1 \times 10^9$  cells each).
- Negative and positive selection can be executed simultaneously.
- Sample volume: from 250  $\mu$ L to 8.5 mL for each sample.

#### USER INTERFACE:

- Intuitive graphical user interface.
- Integrated 8" TFT color touch-screen.
- Pre-programmed protocols for all EasySep™ selection kits.
- Stores and reports user ID, sample ID and reagent lot numbers.
- Software and procedures can be updated, and usage data saved, through the Ethernet or USB connections.
- Laboratory automation: can be controlled externally using XML-RPC for integration into automated processes.
- Connections: RJ-45 10/100 Ethernet port, 2 USB ports.

#### TECHNICAL SPECIFICATIONS:

##### Dimensions:

- Height with lid: 56 cm (21 $\frac{7}{8}$ " )
- Width: 71 cm (27 $\frac{7}{8}$ " )
- Depth: 39 cm (15 $\frac{3}{8}$ " )
- Weight: 26 kg (57 lb)

**Power Requirements:** 50/60 Hz, AC 100-240V.

##### Conditions for Operation:

- Temperature: 10-30°C (50-86°F). RoboSep™ is not specified for use in a cold room (4°C, 39°F).
- Humidity 20-85% (non-condensing).

## RoboSep™ Parts & Accessories

| PRODUCT NAME  | CATALOG # |
|---|-----------|
| RoboSep™ Service Rack   | 20101     |
| Hydraulic Fluid Bottle  | 20102     |
| RoboSep™ Buffer (250 mL) <sup>1</sup>                           | 20104     |
| RoboSep™ Buffer 5X Concentrate (250 mL)                         | 20124     |
| RoboSep™ Filter Tip Racks (1 box of 8 racks) <sup>1</sup>       | 20125     |
| EasySep™ RBC Lysis Buffer 10X Concentrate (100 mL) <sup>2</sup> | 20120     |
| RoboSep™ Tip Head Cleaner (7 mL)                                | 20119     |
| RoboSep™ Carousel   | 20107     |
| “The Big Easy” EasySep™ Magnet                                  | 18001     |
| Limited Warranty  | 20200     |
| Limited Warranty Plus Preventative Maintenance                  | 20202     |
| Preventative Maintenance Package                                | 20203     |

1. 1 - 2 boxes of RoboSep™ Filter Tip Racks (each containing 8 individual racks) and 1-2 bottles of RoboSep™ Buffer are included with every purchase of a RoboSep™ Filter Tip Reagent Kit.

2. 10 mL of 10X RBC Lysis Buffer is included with every purchase of an EasySep™ or RoboSep™ Whole Blood Selection Kit.