



## Application Note

# WBC depletion using the RosetteSep™ cocktail prior to Parsortix™ separation

## Introduction

**The procedures described in this document are for RESEARCH USE ONLY and not for use in clinical or diagnostic procedures.**

Separating a blood sample on the Parsortix™ system allows the harvest of the target cell population (e.g. circulating tumor cells (CTCs)) along with a background of residual white blood cells (WBCs). Typically, when separating healthy normal volunteer (HNV) blood samples using the recommended standard conditions (whole blood drawn into EDTA vacutainers, separation within 48 hours post draw, GEN3D6.5 separation cassette, 99mbar separation pressure), the residual WBC numbers range from 2,000 cells to about 10,000 cells). For several (especially RNA and DNA based) CTC identification methods these WBC numbers result in a high background noise and make identification and analysis of low numbers of CTCs extremely difficult or impossible.

A very effective way to reduce residual WBC numbers in the Parsortix™ harvest is to deplete blood cells prior to Parsortix™ separation using the RosetteSep™ Human CD45 Depletion Cocktail. The following description of the RosetteSep™ Human CD45 Depletion Cocktail can be found on the vendor's (STEMCELL Technologies) website:

"The RosetteSep™ Human CD45 Depletion Cocktail is designed to enrich epithelial circulating tumour cells (CTCs) from whole blood by depleting CD45+ cells. Unwanted cells are targeted for depletion with Tetrameric Antibody Complexes recognizing CD45, CD66b and glycoporphin A on red blood cells (RBCs). When centrifuged over a buoyant density medium such as Lymphoprep™ (Catalogue #07801), the unwanted cells pellet along with the RBCs. The purified epithelial tumour cells are present as a highly enriched population at the interface between the plasma and the buoyant density medium."

Using the RosetteSep™ Human CD45 Depletion Cocktail prior to sample separation on the Parsortix™ system will reduce the residual WBC numbers in harvests from the typical 6000 cells to between about 30 to 200 cells. However, target cell capture and consequently harvest is also affected by the RosetteSep™ Human CD45 Depletion Cocktail. **Capture is reduced by about 33%**. Overall processing time (from first moment of handling the blood sample until harvest from the Parsortix instrument) is faster and more consistent using the RosetteSep™ depletion cocktail (about 100 minutes). This method is recommended when high levels of purity are required. Additional costs per sample are £30 - £39, depending on ordering low volumes or big bulk orders. The majority of the costs derives from the antibody cocktail (£26.50 - £33).

## Reagents and Consumables

In addition to reagents in PR1-OM-D Parsorter PR1 Operating Manual (needed for standard separation conditions):

Product	Catalogue Number	Manufacturer
RosetteSep™ Human CD45 Depletion Cocktail	15162	STEMCELL Technologies
Lymphoprep™	07801	STEMCELL Technologies
SepMate™-50 (IVD)	85450	STEMCELL Technologies

## Methods

1. Collect blood into 10ml K<sub>2</sub>EDTA Vacutainer tubes and store at 4°C for maximum 48 hours.
2. On the day of blood separation ensure that the Parsortix™ instrument(s) is (are) clean (a cleaning process must always be run after the last separation, harvest or staining process). If in doubt, the instrument(s) should be cleaned again before separating a blood sample.
3. Warm blood samples to room temperature on a roller mixer for 15 minutes.
4. OPTIONAL: If HNV blood is being separated and spiking-in of cancer cells is required, spike each blood sample with 200 CellTracker Green labelled cells following laboratory own procedures. If spiking is not required continue with point 5.
5. Add 50 µl of the RosetteSep™ depletion cocktail per 1 ml of blood to each blood sample (i.e. add 500 µl of the RosetteSep™ depletion cocktail to a 10 ml blood sample).
6. Incubate samples on a roller mixer for 20 minutes at room temperature.
7. Fill 50 ml SepMate™ conical tubes with at least 12 ml Lymphoprep™ reagent. Take care to completely fill the lower chamber of each tube by pipetting through the hole in the center of the tubes.
8. Using a serological pipette layer the blood samples over the Lymphoprep™ reagent.
9. Carefully transfer the SepMate™ tube to a centrifuge and spin at 1200 x g for 20 minutes with the brake off.
10. Remove the tubes from the centrifuge. Using a 10 ml serological pipette, take off the plasma layer from the upper chamber of each SepMate™ tube and transfer it into a standard 50 ml conical tube. Use a pipettor with a 1 ml pipette tip to transfer any remaining plasma layer from the upper chamber into the same 50 ml conical tube. The target cells will be in the plasma layer.
11. Separate and harvest the recovered plasma samples on Parsortix™ instruments using standard separation conditions (i.e. PBS in the buffer reservoir, ethanol in the priming reservoir, 10% Decomatic or 10% LabKlenz 110 in the cleaning reservoir and a GEN3D6.5 cassette) and the following protocols:
  - PX2\_PF (priming)
  - PX2\_S99F (separation)
  - PX2\_H (harvest)

PX2\_CT (cleaning)

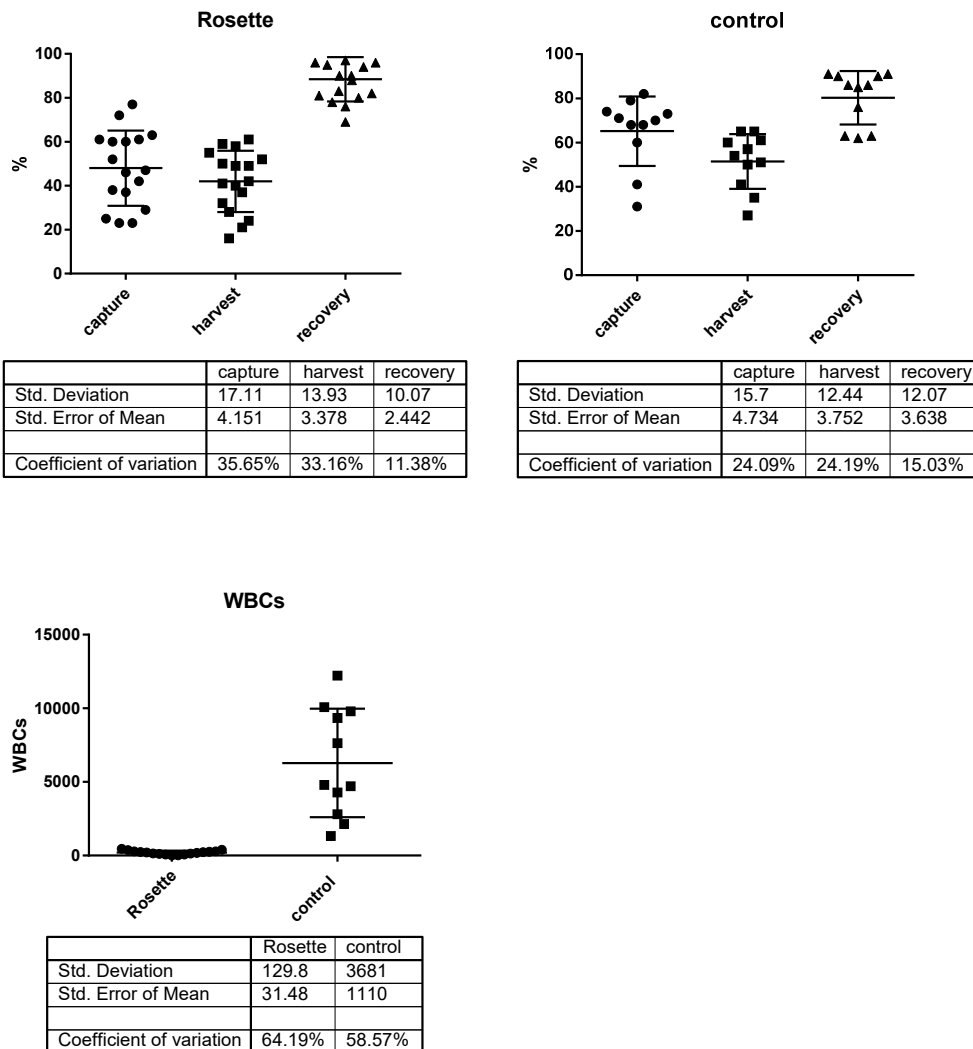
**Note: pre-harvest flush (PX2\_CT2) is NOT required in this procedure**

12. For un-spiked samples identify CTCs in the harvest following relevant protocols. For spiked samples record captured cancer cells in the separation cassette prior to harvest, then record CTCs and WBC numbers in the Parsortix™ harvest.

### Sample Outcomes

This WBC depletion technique has been validated using HNV blood spiked with two cell lines: A549 (lung) and SKBR-3 (breast). In the validation experiments, a minimum of 15 blood samples were each spiked with ~200 cells (for each cell line) and cell capture, harvest, recovery and residual WBC numbers were recorded to assess the overall performance.

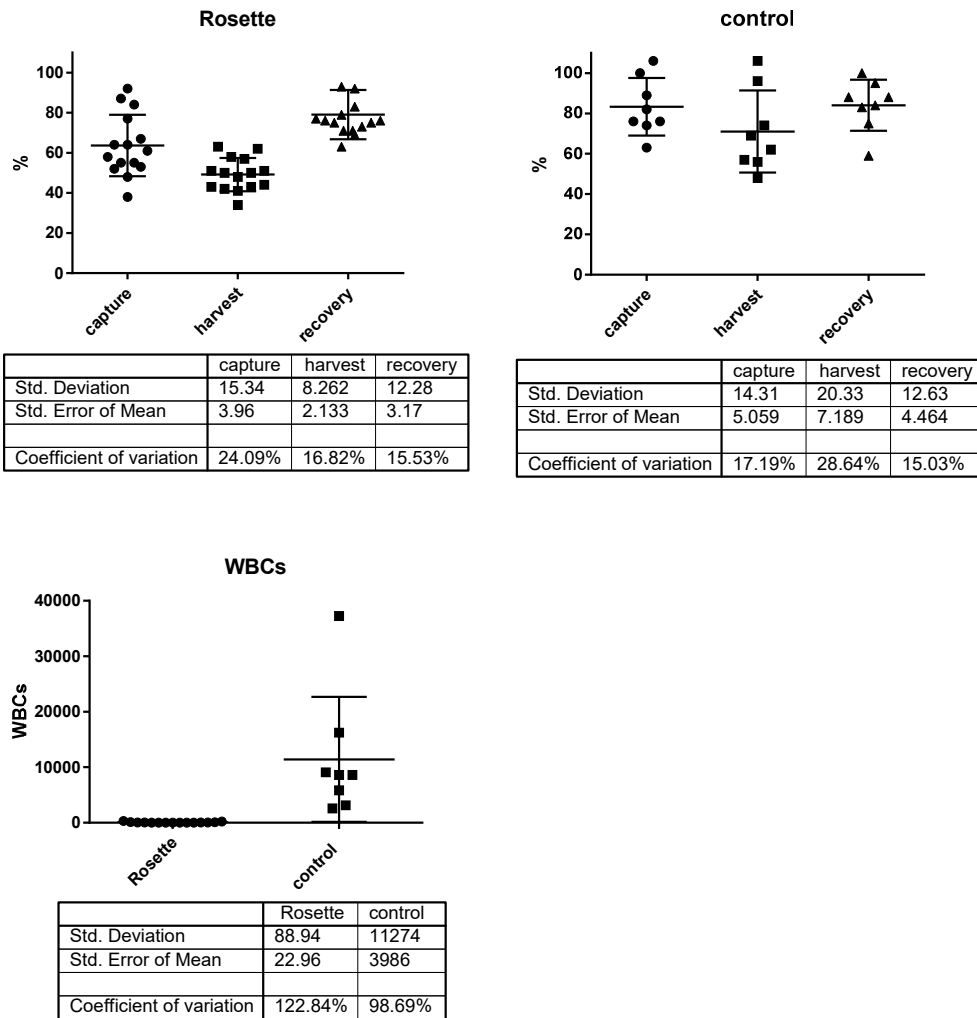
#### Performance with A549 cells



**Figure 1: Sample processing on the Parsortix™ system with and without WBC depletion when using A549 spiked HNV blood. 10 ml EDTA blood samples were spiked with CellTracker Green labelled A549 cells and either treated with the RosetteSep™ depletion cocktail (Rosette) to**

reduce WBC numbers (as described above) or separated on the Parsortix™ instrument immediately after spiking (control). The capture (expressed as percentage of A549 cells caught inside the separation cassette compared with cell spike) and the harvest were enumerated (expressed as percentage of A549 cells in the harvest compared with cell spike). Recovery was calculated as percentage of A549 cells in the harvest compared with A549 cells caught inside the separation cassette. WBC values are total numbers of nucleated blood cells in the harvest. It is worth noting that the variation around the spiked mean of the A549 cells is also reflected in the variation of A549 cell capture and harvest. The data set comprises separations from six separate days.

**Performance with SKBR-3 cells**



**Figure 2: Sample processing on the Parsortix™ system with and without WBC depletion when using SKBR-3 spiked HNV blood.** 10 ml EDTA blood samples were spiked with CellTracker Green labelled SKBR-3 cells and either treated with the RosetteSep™ depletion cocktail (Rosette) to reduce WBC numbers (as described above) or separated on the Parsortix™ instrument immediately after spiking (control). The capture (expressed as percentage of SKBR-3 cells caught inside the separation cassette compared with cell spike) and the harvest were enumerated (expressed as percentage of SKBR-3 cells in the harvest compared with cell spike). Recovery was calculated as percentage of SKBR-3 cells in the harvest compared with SKBR-3 cells caught inside the separation cassette. WBC values are total numbers of nucleated blood cells in the harvest. It is worth noting that

*the variation around the spiked mean of the SKBR-3 cells is also reflected in the variation of SKBR-3 cell capture and harvest. The data set comprises separations from three separate days.*

## Troubleshooting

- Using the RosetteSep™ Human CD45 Depletion Cocktail will lead to cell loss (about 33% for both tested cell lines) compared to the control conditions without RosetteSep™ step. It has not yet been investigated at which step the cell loss occurs. Possible reasons could be target cell loss during the density centrifugation step, target cell loss during transfer steps using pipettes, or target cell loss inside the Parsortix system.
- Care should be taken to transfer the complete plasma layer from the upper chamber without disturbing the density gradient medium when transferring it into standard 50 ml conical tubes. Varying volumes of plasma layer left behind could cause inconsistent capture and harvest results.
- This technique has not been tested with clinical samples.