CELLS FOR PRECISION MEDICINE.

Application Note

Plasma removal from blood sample to enable simultaneous isolation of cfDNA and CTCs

Introduction

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

Use of the Parsortix™ instrument is recommended with whole blood collected in EDTA vacutainers. However, it is useful in some situations to analyse biomolecules, including cfDNA and proteins, in addition to the CTCs. This Application Note describes a procedure to make this possible by removal of plasma before blood separation. The procedure involves centrifugation and aspiration of the plasma, followed by resuspension of the blood cell pellet in buffer prior to separation on the Parsortix instrument.

Note that the capture efficiency of target CTCs is not affected by the procedure, but it does cause a modest increase in the number of white blood cells (WBCs) in the harvest.

Reagents and Consumables

Products	Catalogue Number	Manufacturer
BSA	M6250	Sigma Aldrich
EDTA	74004	Qiagen

Methods

Plasma removal

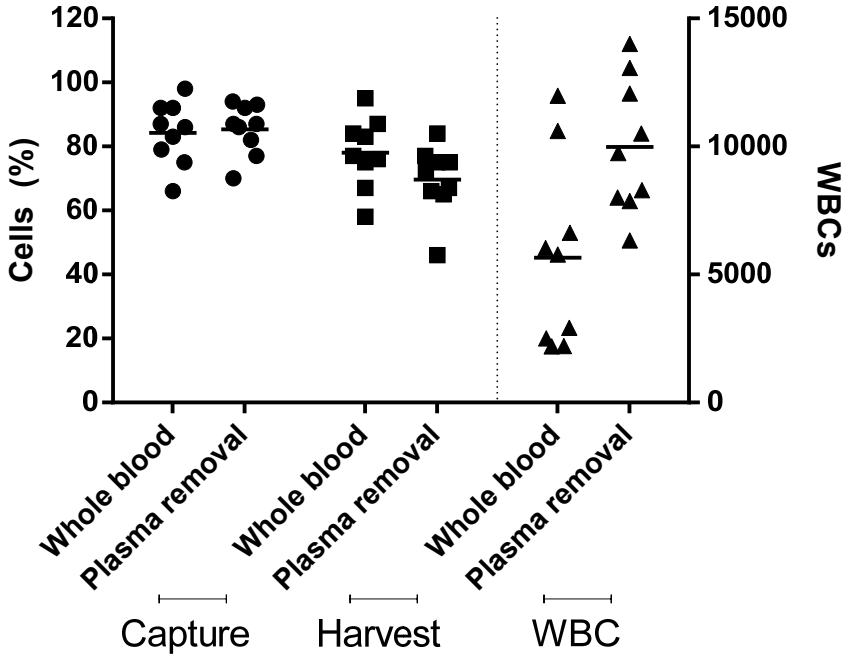
1. Following collection in BD EDTA Vacutainers (BD reference number 367899), centrifuge the blood sample at 2000 g for 10 min (with brake set to 1 or 0).
2. Remove plasma with Pasteur pipette and store as required.
3. To the Vacutainer add 1% BSA (w/v) 2 mM EDTA in PBS to the original volume of 10 ml.
4. Invert tube five times to resuspend blood cells.

Parsortix™ separation and harvest

1. Following the user manual instructions, separate the blood sample and proceed to downstream analysis as required.

Sample Outcomes

This technique has been validated using the lung cancer cell line A549.



Capture	A549 Whole Blood	A549 Plasma Removal
Mean	84.22	85.33
Std. Deviation	9.795	7.906

Harvest	A549 Whole Blood	A549 Plasma Removal
Mean	78.00	69.67
Std. Deviation	10.99	10.75

WBC	A549 Whole Blood	A549 Plasma Removal
Mean	5651	9986
Std. Deviation	3654	2619

Figure 1: Comparison of separations and harvests of whole and plasma removed blood spiked with A549 cells. *200 CellTracker Green labelled A549 cells were spiked into 10 ml volumes of whole blood (EDTA vacutainers), and either separated whole or after removal of plasma as described above. Cell capture, harvest, and WBCs present in the 210 µl harvests were enumerated. Data comprises triplicate experiments performed on three separate days.*

Cautionary note

- The procedure has not been tested with clinical samples.