

# Direct isolation of untouched human neutrophils from whole blood in as little as 25 minutes

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## Introduction

Neutrophils are the first line of defense against microbial infections. They sense pathogen-associated molecular patterns through a wide range of receptors including toll-like receptors. Once activated, neutrophils eliminate infection via the phagocytosis of pathogens, releasing proinflammatory cytokines and antimicrobial peptides and producing reactive oxygen species (ROS). The current procedures to enrich neutrophils from human whole blood involve at least two steps to remove red blood cells (RBCs) and unwanted leukocytes. For example, neutrophils may be separated from other leukocytes using density gradient centrifugation. However, they are still mixed with RBCs which then need to be lysed. Alternatively, RBCs are first depleted using lysis or sedimentation followed by fluorescence-activated cell sorting (FACS) or magnetic cell separation to remove unwanted leukocytes. Here we describe a one-step immunomagnetic, column-free negative selection method which does NOT require RBC lysis, sedimentation or density gradient centrifugation. Non-neutrophils are targeted for removal with antibody complexes recognizing unwanted cells, including RBCs and platelets. Unwanted cells are labeled with antibodies and magnetic particles and separated using an EasySep™ magnet. Desired cells are then simply collected into a new tube. The complete procedure takes 25 minutes. The purity of isolated neutrophils is  $97.3 \pm 1.4\%$  ( $n = 14$ ). The isolated neutrophils are functional and produce ROS upon activation with PMA. This fast isolation method of untouched, quiescent neutrophils facilitates the studies of neutrophil function in inflammatory diseases.

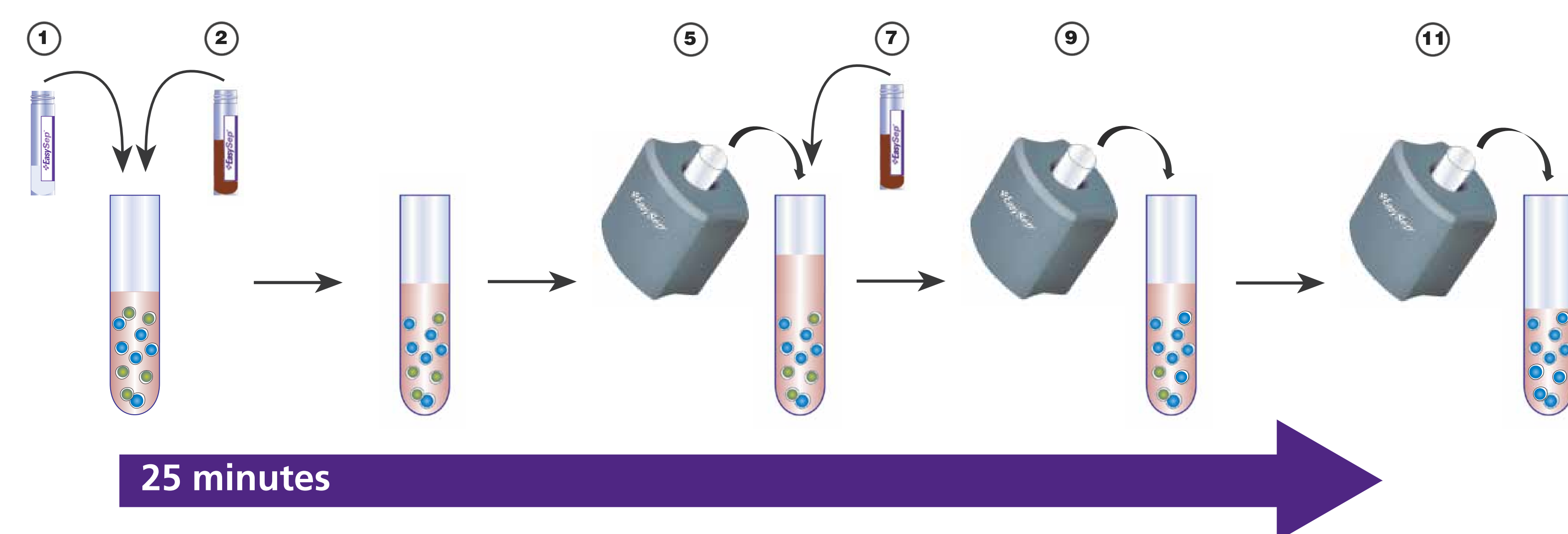
## Methods

For maximum cell recovery, human whole blood was collected using EDTA as an anticoagulant. If non-EDTA based anticoagulants were used, EDTA was added at 1mM to the whole blood. The procedure for EasySep™ Direct neutrophil isolation (Catalog #19666) starting with unprocessed whole blood is outlined in Figure 1.

### Assessing enriched cells

The purity of neutrophils (CD66b<sup>+</sup>CD16<sup>+</sup>) can be measured by flow cytometry after staining with fluorochrome-conjugated anti-CD45, CD66b and CD16. Dead cells are gated out using 7-AAD staining and scatter profile. The purity of neutrophils is assessed after gating on CD45<sup>+</sup> cells.

**FIGURE 1: EasySep™ Direct procedure for column-free isolation of neutrophils directly from unprocessed whole blood**

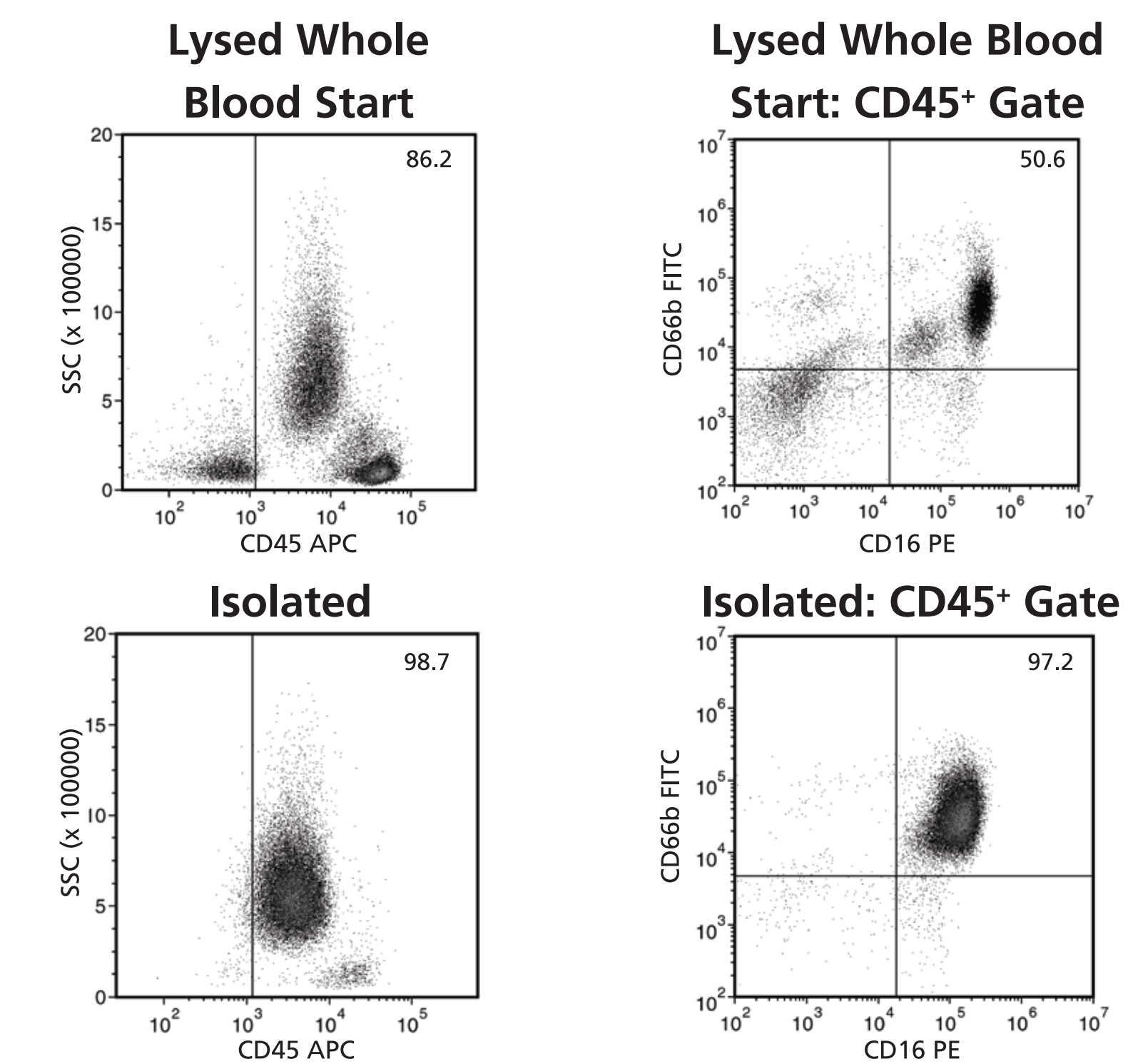


- 1) Add EasySep™ Direct Neutrophil Isolation cocktail
- 2) Add EasySep™ Direct RapidSpheres
- 3) Mix and incubate for 5 minutes at room temperature
- 4) Top-up sample with PBS + 1 mM EDTA
- 5) Place sample into the EasySep™ Magnet and incubate for 5 minutes at room temperature
- 6) Pour sample into a new tube
- 7) Add EasySep™ Direct RapidSpheres

- 8) Mix and incubate for 5 minutes at room temperature
- 9) Place sample into the EasySep™ Magnet and incubate for 5 minutes at room temperature
- 10) Pour sample into a new tube
- 11) Place sample into the EasySep™ Magnet and incubate for 5 minutes at room temperature
- 12) Pour sample into a new tube

## Results

**FIGURE 2: Phenotypic analysis of EasySep™ Direct isolated neutrophils (CD66b<sup>+</sup>CD16<sup>+</sup>)**

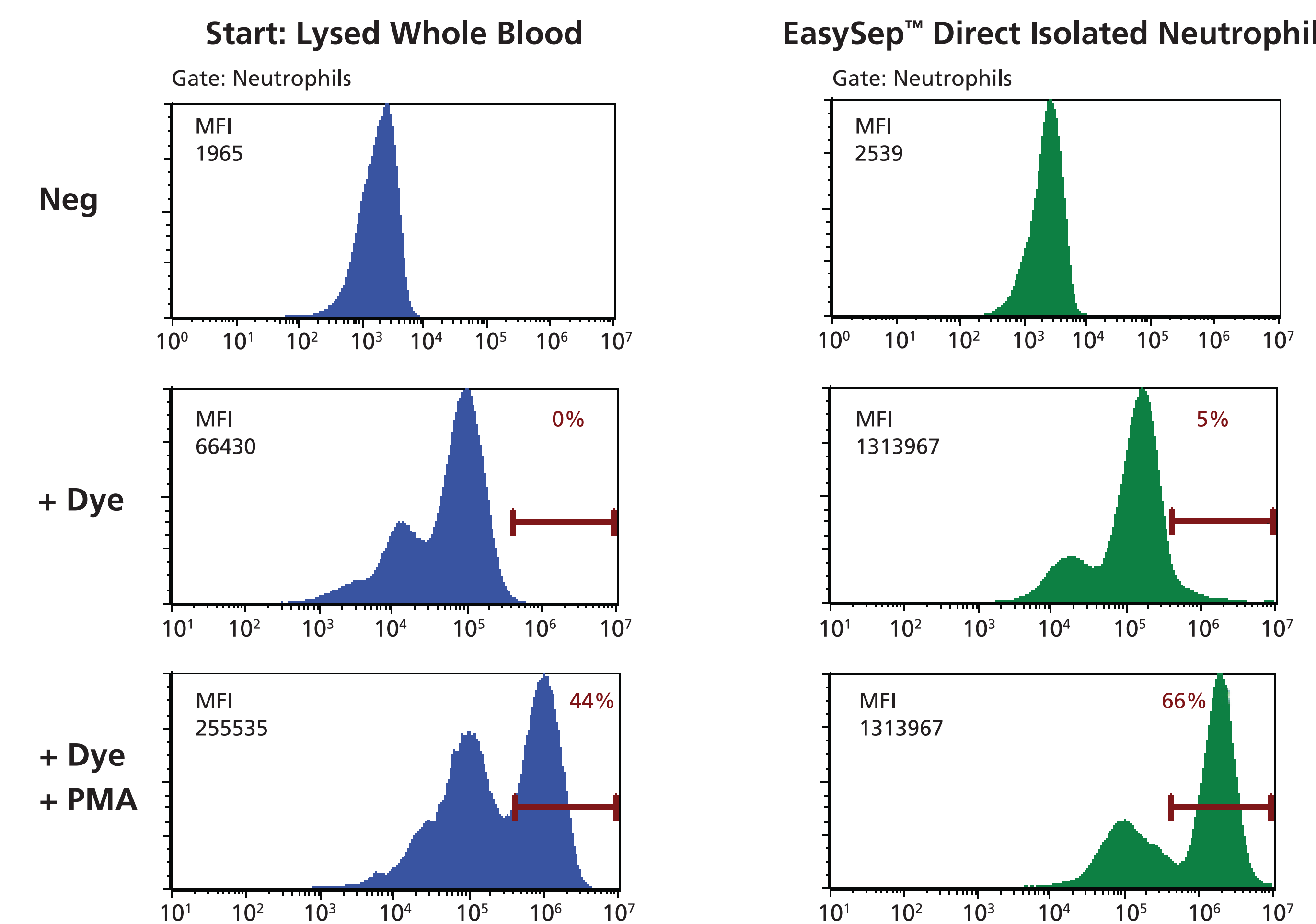


**TABLE 1: Purity and recovery of EasySep™ Direct isolated neutrophils**

Method	n	% Start	% Purity of Isolated Neutrophils	# of Neutrophils Recovered per 1 mL of blood
EasySep™ Direct Neutrophil Isolation	14	55.1 ± 12.5	97.3 ± 1.4	0.6 - 2.9 x 10 <sup>6</sup>

Purities (CD66b<sup>+</sup>CD16<sup>+</sup> gated on CD45) determined by flow cytometry. Values are expressed as means ± SD.

**FIGURE 3: EasySep™ Direct isolated neutrophils produce ROS after PMA stimulation**



PMA induces ROS formation by neutrophils, which in turn results in oxidation of 2,7-Dichlorodihydrofluorescein diacetate (DCFH) into the highly fluorescent product, dichlorofluorescein (DCF). Lysed whole blood cells or EasySep™ Direct isolated cells were resuspended at  $1.25 \times 10^6$  cells per mL in Hanks' Balanced Salt Solution (HBSS) with Ca<sup>2+</sup> and Mg<sup>2+</sup> and 2.5% FBS. Samples were incubated at 37°C for 15 minutes with or without DCFH and washed with HBSS. Cells were then stained with viability dye (7-AAD) and CD16 and CD11b for 15 minutes at 4°C followed by another HBSS wash. Finally, 100 ng/mL PMA was added to the indicated samples and all samples were incubated at 37°C for 25 minutes. Flow cytometry analysis was conducted to determine the formation of DCF in neutrophils as the indicator of DCFH oxidation.

## Conclusions

- Untouched neutrophils can be directly isolated from unprocessed whole blood in 25 minutes.
- More than 99.9% of RBCs are depleted without the need for density gradient centrifugation, sedimentation or lysis.
- Purities up to of 99% neutrophils can be achieved.
- Isolated cells are not activated and are functional as evidenced by ROS production upon PMA stimulation.