# Rapid, one-step isolation of granulocytes directly from whole blood without RBC lysis, sedimentation or density gradient separation

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## Introduction

Granulocytes are polymorphonuclear cells (PMNC) containing cytoplasmic granules. The three types of granulocytes – neutrophils, eosinophils and basophils – are distinguished by their appearance under Wrights' stain. Neutrophils (70 - 80% of all granulocytes) are a first line of defense against microbial infections. Eosinophils (10 - 15% of all granulocytes) play an important role in neutralizing parasites, while basophils (least abundant) contribute to the immune response during allergy. To isolate purified granulocytes from whole blood, both red blood cells (RBC) and mononuclear cells (MNC) must be removed. Traditionally, either the RBC are first removed using ammonium chloride lysis or hetastarch sedimentation and then the granulocytes are immunomagnetically selected, or the granulocytes are separated from unwanted MNC via density gradient centrifugation and then the RBC/PMNC pellet is lysed to recover the granulocytes. Both methods typically take >1 hour. We have developed a one-step method to isolate granulocytes directly from whole blood in 25 min without RBC lysis, sedimentation or density gradient centrifugation. Unwanted MNC, platelets and RBC were immunomagnetically labeled for 10 min and the sample placed in an EasySep™ magnet for 5 min. Magnetically labeled cells were retained in the magnet and the untouched granulocytes were simply poured or pipetted off. The magnetic incubation and pour- or pipette-off steps were repeated twice more. The purity of enriched pan-granulocytes was 98.4 ± 1.5% (n = 29) and on average 1.6 x $10^6$ granulocytes were recovered per mL of whole blood. There was no difference in the purity of granulocytes thus enriched compared to lysis followed by immunomagnetic negative selection ($p > 0.05$, n = 29). This method to rapidly isolate untouched pan-granulocytes directly from whole blood with no additional cell processing steps will aid the study of the roles the different granulocytes play in the fight against pathogens.

## Methods

### Cell Isolation

**EasySep™ Direct Pan-Granulocyte Isolation Kit (Cat. #19659):** Granulocytes were isolated from whole blood according to the Product Information Sheet for Catalog #19659.

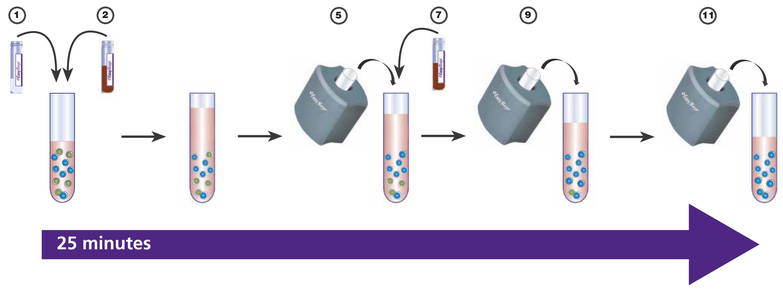


**Figure 1:** EasySep™ Direct procedure for column-free isolation of pan-granulocytes from unprocessed whole blood.

1) Add EasySep™ Direct Pan-Granulocyte 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, cells are now ready for downstream use

**EasySep™ Pan Granulocyte Enrichment Kit (Cat. #19259):** Granulocytes were isolated from ammonium chloride lysed whole blood according to the Product Information Sheet for Catalog #19259.

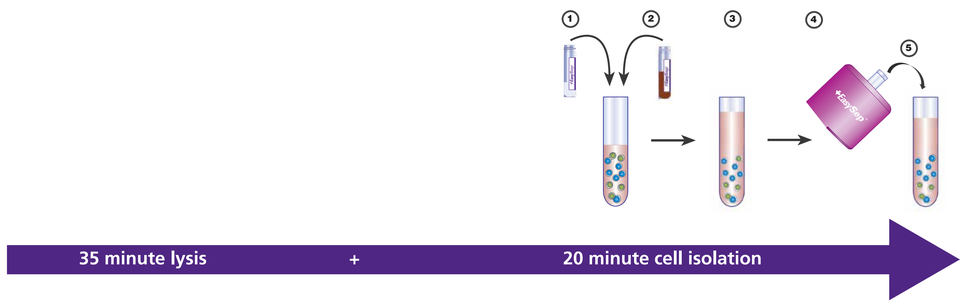


**Figure 2:** EasySep™ procedure for column-free isolation of pan-granulocytes from lysed whole blood.

1) Add EasySep™ Pan-Granulocyte Isolation cocktail and incubate for 10 minutes at room temperature
2) Add EasySep™ D2 Magnetic Particles and incubate for 5 minutes at room temperature
3) Top-up sample with 2% PBS + 1 mM EDTA
4) Place sample in the EasySep™ Magnet and incubate for 5 minutes at room temperature
5) Pour sample into a new tube, cells are now ready for downstream use

### Assessment of Isolated Cells

The purity of neutrophils ($CD66b^+CD16^+$), eosinophils ($CD66b^+CD16^-$) and basophils ($CD66b^-CD123^+$) was measured by flow cytometry after staining with fluorochrome-conjugated antibodies against CD45, CD66b, CD16 and CD123 (Figure 3). Dead cells were gated out using 7-AAD staining and scatter profile. The purity of all the granulocyte populations was assessed after gating on $CD45^+$ cells.

## Results

| Isolation Kit | n | Purity of Granulocytes | Time to Isolate Granulocytes from Whole Blood (min) |
|---|---|---|---|
| EasySep™ Direct Pan-Granulocyte Isolation Kit (Cat. #19659) | 29 | 98.4 ± 1.5 | 20 or 30 (depending on magnet) |
| EasySep™ Pan Granulocyte Enrichment Kit (Cat. #19259) | 29 | 98 ± 1.6 | 55 |

Purities determined by flow cytometry. Values are expressed as mean ± SD.

**Table 1:** Purity of pan granulocytes (neutrophils, eosinophils and basophils) isolated from whole blood using either the new EasySep™ Direct Pan-Granulocyte Isolation Kit (Cat. #19659) or the EasySep™ Pan Granulocyte Enrichment Kit (Cat. #19259). There is no significant difference between the purity of pan-granulocytes isolated with either method.

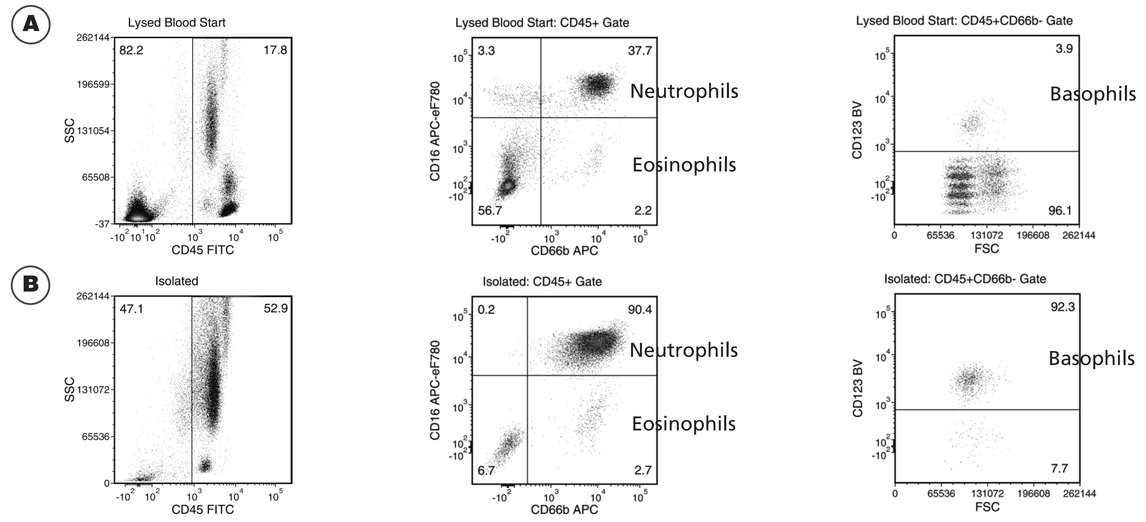


**Figure 3: Typical flow cytometry plots for the new EasySep™ Direct Pan-Granulocyte Isolation Kit (Cat. #19659).** In this example, the pan-granulocyte content (neutrophils, eosinophils and basophils combined) of the **A)** lysed whole blood start sample and the **B)** non-lysed final isolated fraction is 42.2% and 99.5%, respectively (gated on CD45).

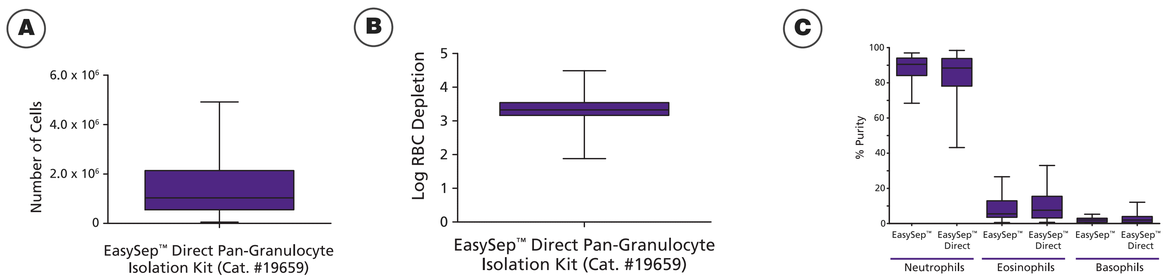


**Figure 4: A)** On average 1.6 ± 1.4 x $10^6$ (n = 29) granulocytes (neutrophils, eosinophils and basophils) are recovered from 1 mL of whole blood using the new EasySep™ Direct Pan-Granulocyte Isolation Kit (Cat. #19659). **B)** On average, the log depletion of red blood cells, as assessed by flow cytometry analysis of $GlyA^+$ cells, following isolation directly from whole blood using the new EasySep™ Direct Pan-Granulocyte Isolation Kit (Cat. #19659) is 3.3 ± 0.5 (n = 16). **C)** Percentage of different granulocyte populations (neutrophils, eosinophils and basophils) isolated using the EasySep™ Pan Granulocyte Enrichment Kit (Cat. #19259) and the new EasySep™ Direct Pan-Granulocyte Isolation Kit (Cat. #19659) (n = 29 for each data set).

## Conclusions

- Untouched granulocytes (neutrophils, basophils and eosinophils) can be isolated directly from unprocessed from whole blood in as little as 25 minutes.
- More than 99.9% of RBCs are depleted without the need for density gradient centrifugation, sedimentation or lysis.
- Purities of 98.4 ± 1.5% can be achieved.