APPLICATION NOTE No. 465 | July 2022

EU-IVD Products

# Optimized Isolation of Mononuclear Cells via Software Controlled Acceleration and Braking Ramp

Holger Heine[1], Thomas Uschkureit[2], Kerstin Isermann[2]
[1]Research Centre Borstel, 23845 Borstel
[2]Eppendorf SE, 22339 Hamburg

## Abstract

The Centrifuge 5702 family offers a reliable alternative to larger multipurpose benchtop centrifuges in clinical and cytological laboratories with applications that do not require g-forces above 3,000 × *g*. The wide range of applications covered by this centrifuge family has been made possible by continuous improvements based on the application requirements of our customers. This includes both improvements to hardware and software features, e.g. the recently launched vacutainer buckets and the implementation of a Timer function.
The Centrifuge 5702 family can be used for more than just pelleting applications.

It is also suited for successful use in the field of gradient centrifugation, such as the isolation of peripheral blood mononuclear cells (PBMNC). This method is based on the application of Ficoll-Paque® density gradient centrifugation in 15/50 mL conical tubes, or blood collection tubes, respectively, and relies on a clean and good separation of PBMCs in sufficient quantity and quality.
The development and subsequent implementation of a software that allows this application to be carried out successfully on such a small device was developed in cooperation between academic research and industrial development.

## Introduction

Many applications in clinical and cytological laboratories rely on rapid processing of samples. Since centrifuges are required in almost every workflow, they can contribute significantly to the optimization of routine processes. The continuous development and adaption of centrifuges to meet the specific applicative requirements of the customer is an important element in achieving this. This includes improvements to both, software and hardware features, such as enabling the accommodation of certain types of tubes. With the models Centrifuge 5702, 5702 R and 5702 RH, Eppendorf offers centrifuges for low to mid-throughput applications, e.g. in cell culture, clinical trial, or other laboratories. As time is precious in every lab, this dedicated centrifuge family was developed both with a short acceleration and fast braking time (with an average of 19 sec. – depending on centrifuge model and rotor). This feature does not only enable time savings in routine workflows, e.g. through faster harvesting, but also prevents possible degeneration of samples, as these do not remain in the centrifuge longer than necessary.
However, speed is only one aspect of working in the lab. Especially when it comes to sensitive applications like density gradient centrifugation, re-mixing of phases must be prevented and excessive braking speeds (deceleration) can be a disadvantage. For a long time, such applications could only be carried out successfully with larger benchtop centrifuge models that offer adjustable acceleration and deceleration ramps. To enable our customers to successfully perform applications that rely on density gradient centrifugation in the small low-speed Centrifuge 5702 family as well, special attention has been paid to the development of an additional acceleration and deceleration ramp that is gentle yet fast. This Application Note will show how a clinical standard protocol was used to optimize the software for Centrifuge 5702 models by implementing a "Soft function" that enables the user to perform density gradient centrifugation with reliable, reproducible results in the shortest period of time. Countless applications in biomedical routine diagnostics rely on highly viable and functionally intact cell populations. As the isolation of human mononuclear cells via density gradient centrifugation is a standard method to achieve this, this protocol was chosen for our purpose. This specific separation technique is based on the density properties of Ficoll (1). Due to their high density, red blood cells (erythrocytes) pass the Ficoll phase (density: 1,077 g/mL) and form a sediment. Lymphocytes, thrombocytes, and monocytes are collected within the plasma gradient phase (specific weight less than 1,077 g/mL) and thus can be enriched and used either directly for experiments or as a starting population for further isolation of single types of cells.

## Material and Methods

Three different software variants for Centrifuge 5702 were tested. As a reference model the larger benchtop Centrifuge 5810 with swing-bucket rotor A-4-44 and corresponding adapters for 50 mL conical tubes was chosen. This centrifuge has 10 acceleration and braking ramps, whereby level 0 (lowest acceleration and brake) was selected for this study. Isolation of human mononuclear cells (MNC) was carried out in 50 mL conical tubes using a Biocoll separating solution. To evaluate the quality of the separation in this experiment, a visual assessment first determined whether a defined interphase with clearly delineated phase transitions was visible. Subsequently, the number of cells per phase was counted to verify the separation process in terms of quantity. The blood samples used for the experiments originated from one donor to guarantee comparability of the results.

1. 10 mL Biocoll separating solution (contains Ficoll®400) at room temperature is pipetted into a conical 50 mL tube.
2. Human blood is diluted 1:2 with HBSS (Hanks balanced salt solution, Invitrogen).
3. The Ficoll phase is covered with a layer of 35 mL diluted blood. It is essential that blood and Biocoll are not mixed!
4. Centrifugation is performed for 30 minutes at 440 × *g* (1,600 rpm). For Centrifuge 5702 with the Soft function switched on and off (Rotor A-4-38, adapter 5702 734.004 for conical centrifuge tubes). For reference Centrifuge 5810 with ramp setting 0 for both, acceleration and deceleration (Rotor A-4-44, adapter 5804 758.005 for conical tubes).
5. Optical analysis of the resulting gradients using a digital camera.

6. The HBSS/thrombocyte/blood plasma supernatant is carefully pipetted off to approx. 1 cm above the MNC ring.
7. The MNC is carefully transferred to a fresh 50 mL vessel using a 10 mL pipette. All the steps are performed on ice and no more than 20 mL per vessel is processed.
8. HBSS is used to top up the level to 50 mL. The mixture is shaken to mix it thoroughly and centrifuged with the brake switched on (10 min, 1,600 rpm, 440 × *g*).
9 When the supernatant has been removed by pipette, the pellet is resuspended in 1 mL HBSS and step 8 (centrifugation) is repeated.
10. Finally, the supernatant is removed by pipette and the cells are absorbed by 5 mL culture medium. The number of the cells is counted (Neubauer counting chamber) and their size is determined. For this purpose, 50 µL of the cell suspension is mixed with 10 mL counting fluid (Isoton II, Beckmann Coulter, Krefeld) and analyzed in a Coulter Channelyzer 256 (Beckmann Coulter, Krefeld).

## Result and Discussion

The results contained in this experiment were initially documented with regard to quality by the visual assessment of the gradients (see step 5, material and methods). At the end of the experiment, they were verified with regard to quantity by counting the number of cells. Care was taken that each original sample could be used to form at least four gradients: two in the Centrifuge 5702 model being investigated and two in the reference Centrifuge 5810.
First, the Centrifuge 5702 was operated with the brake switched on for all the necessary centrifugation steps. Fig. 1a shows clearly that, besides careful layering of the whole blood and medium mixture on to the Ficoll (see material and methods), the brake setting used in the centrifuge is of great significance for this application. When the brake is switched on, isolation of the mononuclear cells is not possible as no MNC ring can be detected. In the next step, a "Soft function" was implemented in Software I, which resulted in a slightly prolonged acceleration and unbraked stopping of the rotor (Fig. 1b). A comparison of these results with those obtained using the reference Centrifuge 5810 (Fig. 1c) proves that a detectable gradient was formed in both experiments. Nevertheless, the gradient formed in the reference centrifuge was significantly more distinct. This optical impression is confirmed by the cell yield which was 12 % lower than in the reference centrifuge (cf. Table 1).

A significant improvement in the centrifugation results was achieved using Centrifuge 5702 with the further developed software variant II. This variant enables both smooth, slow acceleration and smooth, electronically controlled braking. The comparison shown in Fig. 2 reveals clearly that the ring of mononuclear cells is considerably easier to locate in Centrifuge 5702 with software variant II (Fig. 2b) than in the centrifuge with variant I (Fig. 2a). This results in both a higher cell yield (cf. Table 1) and in a reduction of undesirable cells (e.g. thrombocytes), as shown by means of a stray light analysis (cf. material and methods, results not shown).
In a third step, the software was further optimized (variant III) to enable the braking time to be as short as possible without compromising the successful phase separation. Fig. 3 shows a comparison between software variants II and III. The formation of the Ficoll gradients is comparably good in both cases, with variant III allowing considerably shorter braking times (approx. 60 sec. in comparison with approx. 180 sec. for variant II). These results show that it was possible to develop a soft function for both acceleration and braking that allows reproducible, quick, and clean isolation of mononuclear cells using a Ficoll gradient. This further development of the software has thus considerably expanded the field of application of the Centrifuge 5702 family.


Fig. 1a


Fig. 1b

Fig. 1c

**Fig. 1:** Comparison of the gradient formation between Centrifuge 5702 and reference centrifuge.
a) Centrifuge 5702 with brake switched on.
b) Centrifuge 5702 with Software variant I and Soft function switched on.
c) Reference Centrifuge 5810 with ramp setting 0.


Fig. 2a


Fig. 2b

**Fig. 2:** Comparison of the gradient formation in Centrifuge 5702.
a) Software variant I with Soft Function switched on.
b) Software Variant II with Soft Function switched on.

| Centrifuge | Centrifuge 5702 | | | Centrifuge 5810 |
|---|---|---|---|---|
| | **Software variant I** | **Software variant I** | **Software variant II** | **Reference** |
| **Program for Ficoll gradients** | | | | |
| **rpm; rcf** | 1,700 rpm; 440 × *g* | 1,700 rpm; 440 × *g* | 1,700 rpm; 440 × *g* | 1,600 rpm; 440 × *g* |
| **Time (total)** | 30 min | 30 min | 30 min | 30 min |
| **Brake, acceleration** | Max. | Soft function | Soft function | Min. (ramp 0/0) |
| **Washing program** | | | | |
| **rpm; rcf** | 1,700 rpm; 440 × *g* | 1,700 rpm; 440 × *g* | 1,700 rpm; 440 × *g* | 1,600 rpm; 440 × *g* |
| **Time (total)** | 30 min | 30 min | 30 min | 30 min |
| **Brake, acceleration** | Max. | Max. | Max. | Max. (ramp 9/9) |
| **Amount MNC** | Not detectable | 152 million | 168 million | 172 million |

**Table 1:** Description and results of experiments for determining the optimal soft function.

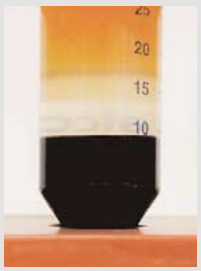


Fig. 3a

Fig. 3b

**Fig.3:** Comparison of the gradient formation. a) Centrifuge 5702 with software variant II, Soft function switched on. b) Centrifuge 5702 with software variant III, Soft function switched on.

## Conclusion

This Application Note shows how cooperation between academic research and industrial development allows the improvement of laboratory devices according to the requirements of a standard application. It enabled the optimization of the Centrifuge 5702 software for the isolation of human mononuclear cells (MNC) from whole blood by means of density gradient centrifugation based on the properties of Ficoll. Although the first improved software variant was suitable for this method, the results could be further improved by optimizing the control software.
In the course of the cooperation, various different software versions for the Centrifuge 5702 were investigated with regard to their suitability for this application. The optimized software that enables obtaining a maximum yield of clean, well-separated and viable MNCs, while further reducing the rotor deceleration time, and was subsequently implemented in the Centrifuge 5702 family. This includes Centrifuge 5702, the refrigerated version Centrifuge 5702 R, and the temperature controlled Centrifuge 5702 RH, that can both heat and cool samples with very precise temperature controlling (+/- 0.5 °C at 4 °C and 37 °C). The "Soft function" is recommended for optimal results in density gradient centrifugation. Together with the recently implemented timer feature of the software and the vacutainer buckets this family of low-speed and small footprint centrifuges is suitable for a great range of low speed applications in clinical routine laboratories.

## Literature

**[1]** Böyum, A. Isolation of mononuclear cells and granulocytes from human bloss. Scand.J.Lab.Clin.Invest 1968; 21:77-89.

| Solution | Components | Ordering no. |
|---|---|---|
|  | **Centrifuge 5702,** without rotor | 5702 000 019 |
| | **Centrifuge 5702 R,** without rotor | 5703 000 012 |
| | **Centrifuge 5702 RH,** without rotor | 5704 000 016 |
| | **Swing-bucket rotor A-4-38,** incl. 4 à 85 mL round buckets | 5702 720 003 |
| | **Adapter for 50 ml Falcon tubes,** set of 2 | 5702 734 004 |
| | **Bucket for 16 x 125 mm round bottom tubes for rotor A-4-38** | 5702 764 000 |
| | **Centrifuge 5810,** without rotor | 5810 000.017 |
| | **Centrifuge 5810 R,** without rotor | 5811 000.010 |
| | **Swing-bucket rotor A-4-44,** incl. 4 rectangular buckets à 100 mL | 5804 709 004 |
| | **Adapter for 50 ml Falcon vessels,** set of 2 | 5804 758 005 |

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