

Integrated Solutions for High-Throughput Bioprocessing Applications Using the Thermo Scientific Sorvall RC12BP Centrifuge

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KEY WORDS

- High Throughput Processing
- Large Volume Sample
- Blood Banking
- Industrial Laboratory
- Low Speed Centrifuge
- Large Capacity Rotor
- 2 Liter Bottle

Introduction

Advances in blood banking, industrial biotechnology, bio-production and pharmaceutical laboratories have driven the need to process multiple sample batches. This application brief describes diverse solutions to improve efficiency and reproducibility of high-throughput (large-volume samples) and low-speed applications using specific Thermo Scientific products including the Sorvall RC12BP low-speed centrifuge, H-1200 high capacity swinging bucket bioprocessing rotor and Nalgene 2 L Bio Bottle.

The Sorvall® RC12BP™ low-speed centrifuge offers high-quality blood product reproducibility and high throughput to meet demanding blood bank processing requirements. When used with the H-12000 swinging-bucket rotor, the Sorvall RC12BP centrifuge enables the ability to run up to 12 blood bags or 12 L of sample in a single run, each with 500 mL (maximum 550 mL) collection volume.

Procedure

Blood Bank Laboratories

PROTOCOL 1: Preparation of Platelet Concentrate from Platelet-Rich Plasma

Following the calibration of centrifuges for platelet manufacture in the AABB Technical Manual, the AABB suggests the following centrifuge conditions¹:

First Spin: Separates whole blood unit into platelet-rich plasma (PRP),

buffy coat (WBCs) and red blood cells (RBCs) by centrifugation at 2000 x g for 3 min.

The first centrifugation step for platelet production is critical. If the first spin is too long and hard, platelets will be lost to the buffy coat and the red blood cells, resulting in low platelet yields. Conversely, too short a run does not provide enough time to separate an adequate plasma volume from the whole blood. This results in a reduced volume of plasma products and bloody platelets.

Second Spin: To obtain the platelet concentrate, the first manufacture of the platelet-rich plasma is followed by the second spin at 5000 x g for 5 min (see Table 1).

During the second spin, platelets are pelleted from the platelet-rich plasma to yield platelet concentrate and platelet-poor plasma (PPP). In choosing the length and speed of the second spin, platelets should be pelleted tightly, but not to the point of aggregation. If this happens, the platelet yield will appear low because the cells stick together and the unit cannot be used. Also, platelet contaminants in the plasma can decrease the antihemophilic factor (AHF) yield; therefore, the plasma should be as cell-free as possible.



Thermo Scientific Sorvall RC12BP Centrifuge

How to Adjust Your Protocol

Make adjustments in the speed by 200 rpm increments or time by 30 seconds. Adjust the protocol until the desired yield of products is obtained.

- If the platelet yield is low or plasma volume is high, decrease the time or lower the speed of the first spin.
- If the plasma yield is low, increase the time or speed of the first spin. (See Table 2 for a Troubleshooting Guide to Improve Blood Product Yields.²)

	H-12000 Blood Processing Rotor	Recommended RCF (x g)	Recommended Speed (rpm)	Spin Time (min.)	Temperature (°C)
Platelets from PRP	1st spin:	2,000	2,455	3	22
	2nd spin:	5,000	3,880	5	22
Platelet from WBC (Buffy Coat)	1st spin:	5,000	3,880	7	22
	2nd spin:	2,000	2,455	3	22
Cryoprecipitate from FFP	1st spin:	4,000	3,471	15	4
	2nd spin:	5,000	3,880	10	4

Table 1. Centrifuge conditions for blood components production when using a Sorvall RC12BP centrifuge with a H-12000 blood processing rotor.

Problem/ Observation	1st Spin Finding	1st Spin Action	2nd Spin Finding	2nd Spin Action
Platelet pellet appears firm, well packed	OK	Keep speed and time as is	OK	Keep speed and time as is
Platelet concentrate has aggregates present	OK	Keep speed and time as is	Too hard	Decrease time or speed
Platelet pellet appears soft, loosely packed	OK	Keep speed and time as is	Too soft	Increase time or speed
Plasma and red cell volume acceptable	OK	Keep speed and time as is	OK	Keep speed and time as is
Plasma volume high, red cell volume low	Too hard	Decrease time or speed	OK	Keep speed and time as is
Plasma volume low	Too soft	Increase time or speed	OK	Keep speed and time as is
Platelet yield and plasma volume acceptable	OK	Keep speed and time as is	OK	Keep speed and time as is
Platelet yield is low and pellet appears firm	Too hard	Decrease time or speed	OK	Keep speed and time as is
Platelet yield is low and pellet appears soft	Too hard	Decrease time or speed	Too soft	Increase time or speed
Platelet yield acceptable, plasma volume low	Too soft	Increase time or speed	OK	Keep speed and time as is
No distinct red cell and plasma line. 'Bloody interface'	Too hard	Decrease slow stop rate	OK	Keep slow stop rate same

Table 2. Troubleshooting Guide to Improve Blood Product Yields



Thermo Scientific H-12000 Bioprocessing Rotor

PROTOCOL 2: Preparation of Platelet Concentrate from Buffy Coat (WBC)

Platelets may also be prepared from buffy coat, a method used mainly in Europe.

First Spin: After centrifugation for 7 min at 5000 x g, whole blood is separated into red blood cells, buffy coat containing the platelets, and platelet-poor plasma. The buffy coat is then separated and further processed to obtain a platelet concentrate.

Either a single buffy coat or 4 to 6 pooled (blood group compatible) buffy coats are diluted with plasma or an appropriate nutrient solution.

Second Spin: To concentrate the platelets, the buffy coat or pooled buffy coats is centrifuged for 3 min at 2000 x g (see Table 1).

Leukocyte-depleted platelets can be prepared by filtration; pre-storage leukocyte depletion is recommended (preferably within 6 hours after recovery). Careful optimization of the centrifugation conditions allow leukocyte-depleted platelets to be produced by the buffy coat method.

PROTOCOL 3: Preparation of Cryoprecipitate

Cryoprecipitated is prepared by thawing fresh frozen plasma (FFP) at temperatures between 1°C to 6°C and then recovering the precipitate.

First spin: preparation of cell-free plasma is prepared from whole blood by hard centrifugation at 4000 x g for 15 min at 4°C. The plasma is frozen at temperatures below -30°C. The frozen plasma is then allowed to thaw at 1°C to 6°C in water bath or in a refrigerator.

Second Spin: To prepare the cryoprecipitate, the thawed plasma is subsequently centrifuged at 5000 x g for 10 min at 4°C. The remaining

cryoprecipitate should be re-frozen within 1 hour.

Note: Cryoprecipitated contains coagulation Factor VIII:C, Factor XIII, fibrinogen, vWF (Factor VIII:vWF), and fibronectin. It is required for patients with clotting disorders (see Table 1).

AHF	Antihemophilic Factor
PPP	Platelet-Poor Plasma
PRP	Platelet-Rich Plasma
RBC	Red Blood Cells
WBC	Buffy Coat / White Blood Cell

Table 3. Standard Abbreviations used in Blood Bank Processing

How To Improve Blood Product

Differences in a full or partial rotor load can cause variations in acceleration times. To ensure quality reproducible separations, choose the Accumulated Centrifugal Effect™ (ACE) function, enter the total Accumulated Centrifugal Effect for the protocol, and the Sorvall RC12BP centrifuge will automatically compensate for such variations by adjusting the run time.

Industrial Laboratories

To support large batch in biotech and pharmaceutical environments, the Sorvall RC12BP centrifuge, when used with the high-capacity H-12000 bio-processing rotor, can spin large volumes with 1 L or 2 L centrifuge bottles. The H-12000 rotor is capable of processing 6 x 1000 mL or 6 x 2000 mL sample loads in one spin; it can be centrifuged up to 7333 x g.

Cell-Culture-Based Vaccine Production

Cell-culture-based technology is robust and reliable and is used in the pharmaceutical industry in vaccine production. Cell culture involves growing Mammalian cells in the laboratory in a nutrient solution. The virus is injected into the cells, and cells and viruses multiply. Then the cells' outer walls are removed and the virus is harvested, purified and inactivated. For cells pelleting, low-speed centrifuge is used at 800 x g for 15 min.

Integrated Solutions for Large-batch Bioprocessing Applications

The Sorvall RC12BP low-speed centrifuge and H-12000 swinging-bucket rotor can be combined with the Nalgene® 2 L Bio Bottle to meet high-volume sample processing requirements. For improved pelleting, the Nalgene 2 L Bio Bottle is purpose-engineered to fit the unique conical shape of the H-12000 anodized, aluminum swinging-bucket rotor. The system greatly increases process efficiency when used with the Sorvall RC12BP centrifuge, enabling scientists to process 12 L of sample in a single run.

Nalgene 2 L Bio Bottles can also be used for gross separation of samples, including bacterial, yeast and tissue isolations.

Sterile, single-use Nalgene 2 L Bio Bottles are also available.

Conclusion

The Sorvall RC12BP centrifuge provides an ideal solution for low-speed and high-throughput applications in blood bank and industrial laboratories, delivering reliable and reproducible results. When used with the H-12000 high-capacity rotor and Nalgene 2 L BioBottle, the Sorvall RC12BP centrifuge provides scientists with an easy-to-use, fully compatible centrifugation solution for accelerated productivity of high-volume sample processing applications.

References

1. AABB Technical Manual. 12th Edition 1996.
2. Schroen, D., Downey, H., and Bailey, J. Establishing Blood Banking Protocols on RC3BP and RC12BP. Application Brief, S00052. Thermo Fisher Scientific.



Nalgene 2 L Bio Bottle



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