

Isolation of Mononuclear Cell Fractions Using Your Sepax™ System

CANCER RESEARCH ★

TRANSLATIONAL MEDICINE

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Abstract

The purpose of this protocol is to provide instructions on how to isolate mononuclear cell fractions of apheresis product, bone marrow, or umbilical cord blood using the Sepax™ system with initial volumes of 30 to 120 mL and selectable final volumes of 8-20 mL or 45 mL. This protocol applies to anyone isolating mononuclear cell fractions from apheresis product, bone marrow, or umbilical cord blood using the Sepax™ System. NeatCell C-Pro is Sepax™ C-Pro application software to isolate the mononuclear cells fraction of blood and cellular products via a density gradient medium.

Introduction

A blood cell with a round nucleus is referred to as a peripheral blood mononuclear cell (PBMC) and include lymphocytes (T cells, B cells, NK cells) and monocytes. PBMC do not include erythrocytes and platelets since they have no nuclei, or granulocytes (neutrophils, basophils, and eosinophils) since they have multi-lobed nuclei. Lymphocytes and monocytes are the most abundant PBMCs. PBMCs can be extracted from whole blood using ficoll. Ficoll is a hydrophilic polysaccharide that separates layers of blood. Gradient centrifugation separates the blood into a top layer of plasma, a middle layer of PBMCs, and a bottom layer of polymorphonuclear cells (such as neutrophils and eosinophils) and erythrocytes.

This protocol is a guide to help you isolate mononuclear cell fractions using your Sepax™ system. You can use these guidelines to isolate fractions of apheresis product, bone marrow, or umbilical cord blood.

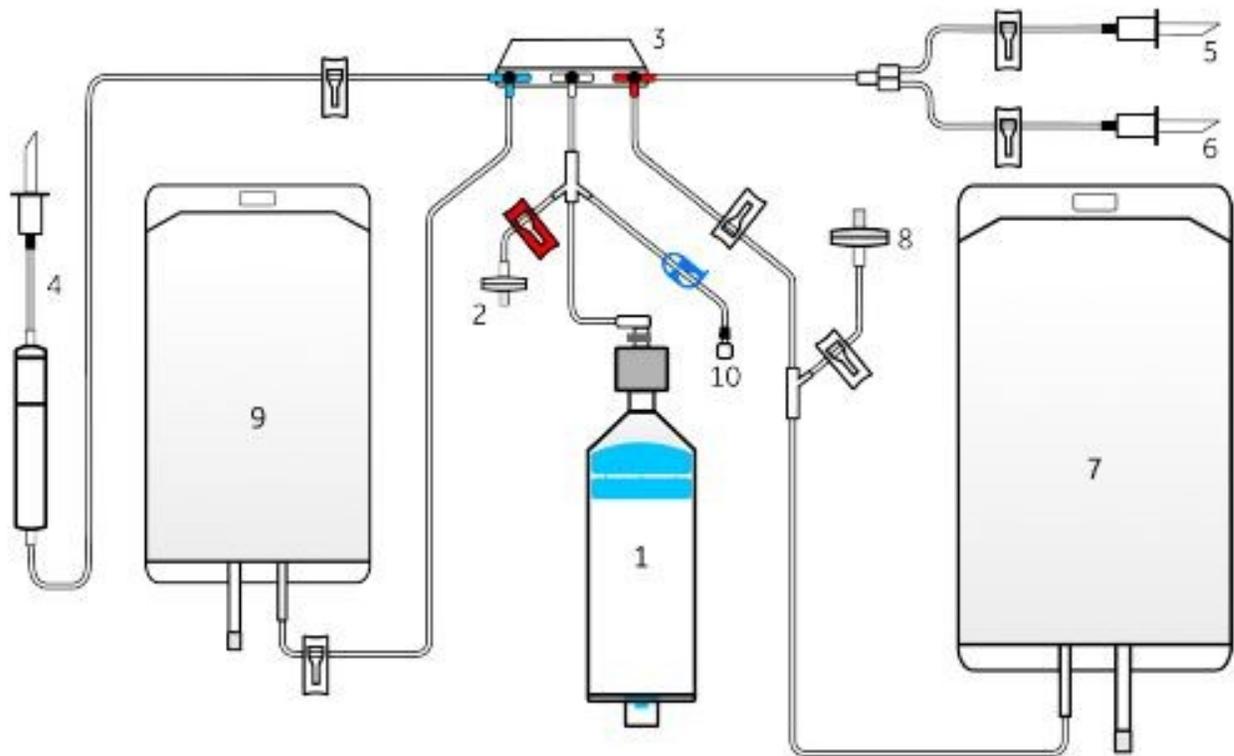
Reagents and Equipment

1. Ficoll
2. Sepax™ Single-Use Separation Kit – CT-90.1
3. BD Syringes (60 mL)
4. Wash Buffer
5. Personal Protective Equipment
 1. Laboratory coat
 2. Safety goggles
 3. Gloves

Procedure

3.1 Kit Preparation

3.1.1 CT-90.1



1	A-220/F separation chamber	Enables centrifugation
2	Pressure monitor	Luer with 0.2 µm bacterial filter to connect to the pressure sensor
3	Stopcock manifold	Three automatically controlled rotational valves to direct fluid flow
4	Initial line	Connects the kit to the initial product bag
5	Washing solution line	Connects the kit to an input solution.
6	Resuspension solution line	Connects the kit to the resuspension solution
7	1000 mL bag	PVC bag to collect by-product
8	DGM Filter	If applicable, bacterial filter for injecting density gradient medium
9	500 mL final bag	PVC bag for the intermediate and final product
10	Final line	Connects the kit to the final bag

3.1.1 In a BSC, remove kit from packaging and inspect for any kinks, ruptures, or visual defects. Ensure packaging was intact, sterility indicator is green, and the expiration date has not passed. Ensure all stopcocks are aligned in the T-T-T position.

3.1.2 Close all 7 clamps, except the red clamp.

3.1.3 If the final volume is between 8 and 20 mL, connect a final container to line 10 using either a sterile tube welder or, under a BSC, a locking luer fitting or spike. If the final container is a syringe, set the parameter "Use syringe for < 20 mL" to

“enabled.”

3.1.4 Connect the washing solution (line 5), resuspension solution (line 6), and the initial bag (line 4) to the kit using either a sterile tube welder or, under a BSC, a spike. Do not thaw the initial product before it is necessary.

3.1.5 Check that the Sepax™ C-Pro is displaying the main application screen. Load the NeatCell application from the menu options.

3.1.6 Open the separation chamber pit and optical sensor covers.

3.1.7 Place the separation chamber into the separation chamber pit and turn the tube output on the separation chamber until it points directly up through the optical sensor towards the stopcock manifold. Firmly press the chamber down.

3.1.8 Place the separation chamber tubing line into the optical sensor and close its cover.

3.1.9 Push down on the two levers to open the holder. Insert the stopcocks into the holder in the T-T position. Close the two levers until they click into place by pulling them up. Ensure the manifold is flat.

3.1.10 Close the separation chamber pit covers and tighten the cover lock by pressing down on it and turning it to the left.

3.1.11 Connect the pressure sensor line (luer connector with filter) to the pressure sensor port on the top of the Sepax™ C-Pro. Tighten it firmly.

3.1.12 Hang the bags: Final and waste bags on the side hooks of the instrument. The washing solution, resuspension, and initial bag on the bag pole holder. Place the drip chamber in its support.

3.2 Sepax™ C-Pro Preparation

3.2.1 Switch the Sepax™ C-Pro ON. It will perform an automatic self-test and display the main menu screen when it is done.

3.2.2 Select and load an application from the main menu screen.

3.2.3 Select *Change parameters*.

3.2.4 Modify parameters as dictated in the Applications section (See 3.3).

3.2.5 If applicable, configure the traceability IDs prior to starting a procedure by selecting *Configure trace ID* in the application menu. Select or disable them as shown below.



1 Configure trace ID

2 Donation ID

3 Operator

4 Initial Bag

5 Final Bag

6 DMSO ID

7 Next

3.2.6 Once finished, press the back button and continue to the desired application.

3.3 Applications

3.3.1 Ensure the source material has a hematocrit level of < 55%, an initial total nucleated cell count of < 100 E6 cells/mL and a total cell volume of < 60% of the total volume. Dilute source material if necessary.

3.3.2 Set the initial volume between 30 and 120 mL (default 50 mL).

3.3.3 Select a final volume. The final volume selection can be set to either a range between 8-20 mL or a fixed final volume of 45 mL. Note that in case of selecting a volume of 8-20 mL, a separate container will be used to extract using line 10 of the kit.

3.3.4 Select the number of washes, either 2 or 3 (default is 2).

3.3.5 If desired, the system can be paused to change the wash buffer for cell re-suspension medium by selecting the *Pause*

switch bag parameter.

3.3.6 For an increased red blood cell depletion, select the *High purity* parameter. Note that this may impact the mononuclear cell recovery.

3.3.7 Set the initial hematocrit value between 0% and 60% (default is 25%).

3.3.8 Select *Start Procedure* and enter traceability IDs if required. After IDs are entered, select *Input done continue* to begin the procedure.

3.3.9 The first step of the process is to manually prime the input line. To manually prime the line, squeeze and release the drip chamber two to three times until it is half full of initial product. Confirm the on-screen message.

3.3.10 Open all four white clamps, except for the clamp with re-suspension solution (if applicable). Confirm the on-screen message.

3.3.11 Lower the initial product bag to the level of the Sepax™ C-Pro and select *Priming* to begin the flow. Press *Stop Priming* when the initial product reaches the point between the blue and white stopcocks on the stopcock manifold. Select *Re-do priming* to repeat the procedure.

3.3.12 If *Pause switch bag* option was selected (Step 4.3.5), the application will pause when the product is in the separation chamber. Close the washing solution line clamp, then open the re-suspension solution clamp. Confirm message and resume the application.

3.3.13 To dismount the kit, follow the screen prompts and remove the bags and air filter. Follow the on-screen prompts to strip the required lines, making sure to hold the line with one hand while stripping.

3.3.14 Close all clamps and dismount the kit, seal the input and output lines.

3.3.15 If a small final volume was selected (Step 3.3.3), remove the air filter and confirm the on-screen message. Remove the stopcocks by following the screen prompt and confirm the message.

3.3.16 Remove the separation chamber and place it upside down on the Separation Chamber Holder C-Pro accessory. Confirm the message on the screen when done.

3.3.17 Close all the clamps, including the red one. Open the blue clamp and use a syringe to push out the cells. Repeat until the piston is at the bottom of the chamber and the cells are in the final bag.

Time Taken

Processing time: approximately 75 min.

Notes and Comments

THIS PROCEDURE IS TO BE PERFORMED BY TRAINED PERSONNEL ONLY.

Abbreviations

PBMC Peripheral blood mononuclear cells

BSC Biosafety cabinet

Waste Disposal

Waste arising from this process should be disposed of via the appropriate waste stream.

For troubleshooting and information on the Sepax™ System, see the User Manual (OM-3074-01).

References

Not available