PRODUCT DESCRIPTION

DACS™SC is a single-use, sterile, prepackaged, kit which contains disposable components sufficient to perform one cell separation. DACS™SC includes the following components:

- Buoyant Density Solution 60 (BDS60), 135 mL
- Separation Container (Diameter 3.8 in., Height 4.75 in.)
- Tubing Set Box, containing
  - Tubing Set A with Luer lock, spike and filtered air vent (1 each)
  - Tubing Set B with Luer lock & spike (1 each)
  - Tubing Set C with 2 Luer locks

All components are terminally sterilized.

PRINCIPLES OF OPERATION

Buoyant Density Solution 60 (BDS60) is a proprietary material containing colloidal silica for use in partitioning of populations of cells in peripheral blood apheresis products based upon their distinctive buoyant densities[1]. The density of BDS60 has been formulated to separate lower density cells, including cells which express the CD34 antigen ($CD34^+$ cells), from higher density cells.

Peripheral blood leukapheresis specimens are layered over the BDS60 solution and centrifuged to separate cells based upon their relative buoyant densities. After centrifugation, $CD34^+$ cells are found in the interface whereas cells with a higher density sediment to the bottom.

DACS™SC contains BDS60 buoyant density solution and a set of plasticware components consisting of a specialized centrifuge separation container and 3 types (A, B, and C) of tubing sets which allow initial processing to occur with minimal exposure to the external environment. The container is compatible with most standard laboratory centrifuges. An insert within the Separation Container facilitates centrifugal cell separation by funneling and trapping high density cells in the bottom chamber of the container.

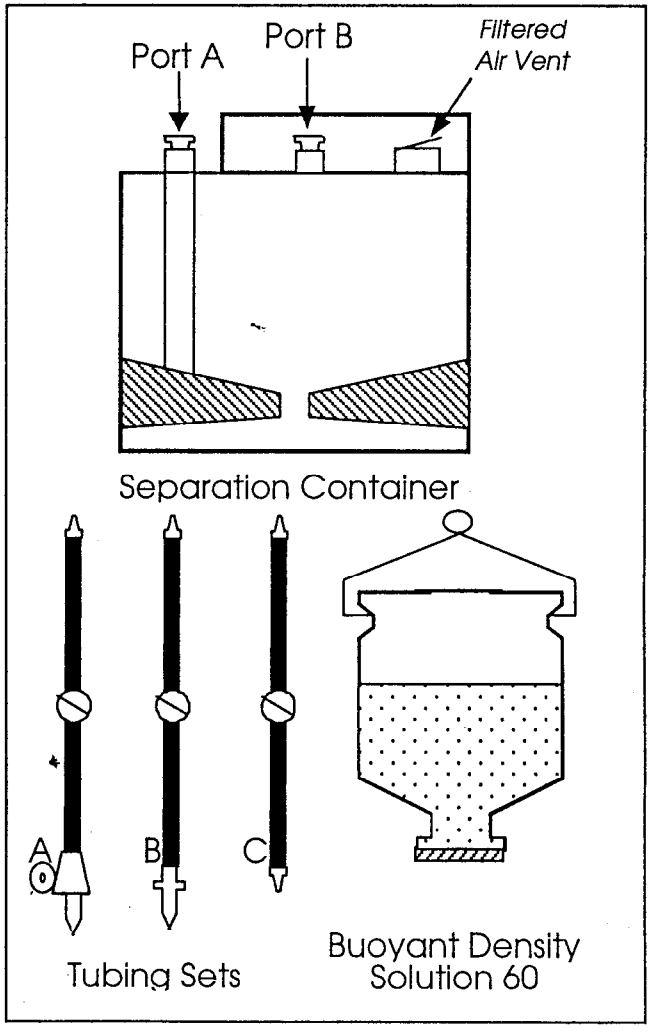


FIGURE 1 DACS™SC components

INDICATIONS AND USAGE

BDS60 is indicated for the processing of autologous mobilized peripheral blood progenitor cells (PBPC) collected by leukapheresis to reduce RBC, platelets and granulocytes in the final PBPC product. BDS60 is not indicated for use with other stem cell products, specifically umbilical cord blood and bone marrow (see **CONTRAINDICATIONS**). Autologous PBPC which have been processed using BDS60 are capable of hematopoietic reconstitution following myeloablative therapy.

It is recommended that sufficient leukapheresis product be collected to provide at least 2 x 10^6 CD34+ cells/kg of patient body weight after BDS60 processing. (See **WARNINGS**).

DEVICE PERFORMANCE

Recovery of CD34 Positive Cells:

The performance of BDS60 solution in recovery of CD34+ cells was evaluated in autologous, mobilized PBPC products obtained from women with breast cancer undergoing PBPC transplantation (see **CLINICAL STUDIES**); data are presented in Table 1. The PBPC products were processed with BDS60 using one of two centrifugation plasticware systems; the DACS™SC kit (n=17) or 50 mL conical centrifuge tubes which contain a cell trap (n=42). The initial median total nucleated cells (TNC) in PBPC processed with DACS™SC was 2.1 x 10^{10} cells (range: 1.2 - 8.1) and for PBPC processed with 50 mL tubes was 2.1 x 10^{10} (range: 1.2 - 6.5). The median recovery of TNC was 32% (range 17 - 52%) after processing with the DACS™SC kit and 42% (range 13 - 78%) after processing with the 50 mL tubes.

TABLE 1. Percent Recovery of CD34 Positive Cells Processed with BDS60 According to Plasticware System

CD34+ cell recovery (%)	DACS™ SC n=17	Conical centrifuge tube n=42
Median	72%	79%
Range	(30-100%)	(34-100%)

After BDS60 processing, the ratio of CD34+ cells to TNC increased by nearly 2-fold (median 1.8 fold with DACS™ SC and 1.9 fold with conical tubes) as compared to the ratio of CD34+ cells to TNC in the initial PBPC product.

Reduction in CD34 Negative Cellular Elements:

The performance of BDS60 processing in reducing other cell populations, specifically TNC, red blood cells, neutrophils, platelets, lymphocytes, and monocytes, was evaluated in mobilized PBPC products from 11 individuals (3 normal donors and 8 with malignancies)[2]. All apheresis products were processed with BDS60 using the DACS™SC plasticware system. The median number of TNC processed was 3.5 x 10^{10} (range 1.2-5.5). The results showed median CD34+ cell recovery of 77% (range: 31-96) with reduction of 37 to 87% in other starting cell populations (Table 2). The clinical significance of reducing RBC, platelets, neutrophils, and lymphocytes was not evaluated.

TABLE 2. Percent Reduction of CD34 Negative Cells With BDS60 (n=11)

Cell type	Median (%)	Range (%)
Total nucleated cells	71	48-91
RBC	87	69-97
Platelets	84	74-93
Neutrophils	82	67-93
Lymphocytes	81	64-91
Monocytes	37	22-90

CLINICAL STUDIES

The clinical safety of the infusion of BDS60-processed, mobilized, autologous PBPC was evaluated in a single arm, two site, clinical trial[3]. Fifty one women with stage II, III, or IV breast cancer underwent mobilization of PBPC for the purpose of autologous transplantation. A total of 59 PBPC apheresis products were collected; of these, 17 PBPC products were processed with BDS60 using the DACS™SC plasticware and 42 were processed using 50 mL conical centrifuge tubes. BDS60-processed PBPC products were cryopreserved in a standard fashion. A total of 46 patients were reinfused with the BDS60-processed PBPC product and were evaluated for infusional toxicity and engraftment profile. The engraftment characteristics of the study subjects were compared to an historical reference group. This reference group consisted of 12 women with breast cancer who had undergone autologous PBPC transplantation at the study sites over a 2 year period immediately preceding the clinical trial. The reference group was selected based on the infused dose of CD34 positive cells ($\geq 2x10^6$/kg) and the progenitor source (peripheral blood).

The median time to neutrophil engraftment for both study subjects and reference group was 9 days. There were no subjects with delayed neutrophil engraftment (ANC >500/mm³ on or after day 14). The median times to platelet engraftment were 9 and 10 days, respectively. One patient each in the study group and the reference group experienced delayed platelet engraftment (platelets >20,000/mm³ on or after day 18). No patient in the clinical trial developed secondary graft failure (Table 3).

TABLE 3. Engraftment Characteristics with BDS60-processed PBPC

	Study population n=46	Reference group n=12
CD34+ cells x 10^6 /kg infused		
Median	6.4	5.6
Range	(2.0-29)	(2.2-67)
Days to ANC > 500/mm³		
Median	9	9
Range	(8-12)	(8-12)
Days to platelets >20,000/mm³		
Median	9	10
Range	(7-19)	(7-25)

CONTRAINDICATIONS

- **BDS60** ***is not formulated for processing bone marrow or cord blood products.***
- **BDS60** ***is not formulated to process previously frozen apheresis products.***

WARNINGS

- *It is recommended that a sufficient number of cells be harvested during leukapheresis to provide for infusion of at least 2 x10⁶ CD34⁺ cells per kg of patient body weight after processing.* Failure to infuse an adequate number of CD34+ cells can result in delayed engraftment of neutrophils and platelets[4], and potentially engraftment failure. Up to 70% of CD34+ cells may be lost during the separation process (see **Device Performance**, Table 1). If at any time the user believes that the target cells necessary for engraftment remain in the nontarget

fraction in the lower Separation Container (see **Harvest and Wash the Cells**, page 8), that fraction may be collected using strict aseptic technique according to the procedure on page 8 (see **Optional Cell Recovery From Lower Separation Container**). **Do not reprocess cells recovered from the lower chamber per the Optional Cell Recovery procedure guidelines on page 8, with the DACS™SC.**

- All components are intended for single use only.
- Do not use bottles and containers with a broken seal or with leakage.

PRECAUTIONS

- Maintain aseptic conditions and perform cell processing using sterile technique.
- Process cells in a biological safety cabinet/laminar flow hood.
- Handle all human specimens as if they are infectious.
- Dispose of all specimens and tubes in compliance with current regulations for biohazardous materials.

ADVERSE EVENTS

There were no clinically significant adverse events or laboratory abnormalities associated with infusion of BDS60-processed PBPC.

Among PBPC products processed with BDS60 using DACS™SC plasticware, there was no evidence of bacterial (n = 17) or fungal (n = 16) contamination after processing. Twenty-six PBPC products processed using 50 mL conical centrifuge tubes were tested for bacterial contamination. One PBPC product that was negative prior to processing became culture positive (*Staphylococcus aureus)* after BDS60-processing. The subject who received these cells did not develop evidence of sepsis.

INSTRUCTIONS FOR USE

General:

- Please read the Critical Considerations section as well as the subsequent instructions completely before starting the separation protocol.
- Ensure that all necessary materials are available.

Critical Considerations for Device Use:

- *Volume of apheresis product.* Do not load more than 250 mL of PBPC sample into the Separation Container.

- *Hematocrit.* Do not load cell suspensions with a hematocrit greater than 20% (>50 mL packed red cells in 250 mL) on the Separation Container. Excess erythrocytes may fill the lower chamber of the Container causing them to overflow into the upper chamber and impairing subsequent cell separation.

- *Temperature.* Performance of BDS60 may be impaired at lower temperatures. Ensure that the temperature of BDS60 is 20° C to 25° C before use.

- *Centrifuge bucket size.* The maximum external diameter of the Separation Container is 3.8 inches (9.7 cm), and external height is 4.75 inches (12.1 cm). Use swinging buckets with a minimum internal diameter >3.8 inches (9.7 cm). Ensure adequate head-space to allow bucket to swing freely.

- *Age of cells.* For best results, process cell samples within 4 hours after collection.

- *Cell clumps.* Do not introduce tissue or cell clumps as they may obstruct the fluid path or interfere with the sedimentation of high density cells in BDS60.

- *Handling of loaded Separation Container.* Handle the loaded Separation Container carefully so that contents in the upper chamber are not mixed.

- *Anticoagulants.* Anticoagulant Citrate Dextrose Solution USP Formula A (ACD-A) is compatible with BDS60 solution. Do not use Heparin or Ethylene Diamine Tetra-Acetic Acid (EDTA) as anticoagulants because they may cause cell clumping.

- *Number of cells processed.* The number of cells that can be effectively prepared using BDS60 is dependent upon the volume of solution and its surface area. There is no minimum number of cells that can be processed but a higher rate of non-specific loss of cells may occur when small numbers of cells are processed (less than 5×10^8 TNC). The maximum number of nucleated cells that yield reproducible results when using the Separation Container is 5×10^{10}. When more nucleated cells are loaded on BDS60, cells may clump resulting in a significant loss of $CD34^+$ cells in the end product.

- *Processing time.* Once the Separation Container is loaded with cells and BDS60, centrifuge immediately. Harvest cells immediately after centrifugation.

- *Preparation and washing solutions.* Use sterile, Ca^{2+} Mg^{2+} free solutions only. Either 1X D-PBS or 0.9% Sodium Chloride Injection USP are acceptable.

- *Centrifugation.* The cells are centrifuged for 30 minutes at 850g at ambient temperature (20-25°C). The time is calculated from the time of acceleration to the time the centrifuge stops. Be sure that the centrifuge is balanced before starting the centrifuge. ***Do not use a brake.*** Braking may result in cell mixing during deceleration.

The number of revolutions per minute (RPM) required to obtain 850g is related to the rotor's radius. Use the nomogram provided to calculate the RPM. To calculate the centrifugal speed, draw a line from the radius measurement through the 850g point in the center column. The centrifugal speed is the point where the extended line crosses the left hand column.

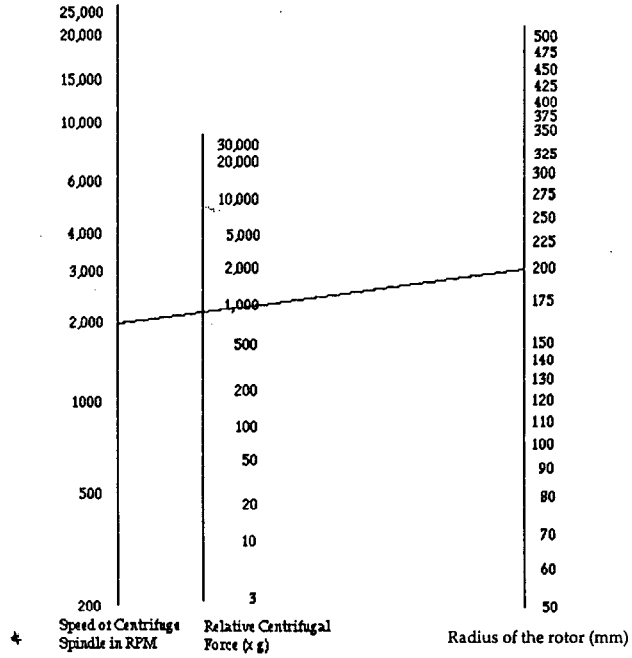
The Nomogram is based on the formula:

$$RCF = 11.18 \times 10^{-7} \times RN^2$$

where:

R = Radius of the rotor (mm)

N = Speed of the spindle in R.P.M.



NOMOGRAM for Computing Centrifugal Speed in R.P.M.

Additional Materials Required:

All media and plasticware must be sterile.

Equipment and reagents required to process cells with DACS™SC:

- Centrifuge with round or square swinging buckets to accommodate the Separation Container with external diameter of 3.8 inches (9.7 cm) and height of 4.75 inches (12.1 cm)
- Laminar flow hood/biological safety cabinet
- Ring stand and clamps
- 0.9% Sodium Chloride Injection USP (Ca^{2+} Mg^{2+} free) or 1X Dulbecco's Phosphate Buffered Saline (D-PBS) (Ca^{2+} Mg^{2+} free)
- 60 cc syringe
- ~~Vortex~~ plasma extractor
- Cell transfer bags with a minimum volume of 600 mL and sterile connector(s). See **HARVEST and WASH Instructions** on page 8.
- Hemocytometer
- Equipment to determine hematocrit

Sample Preparation:

1. Use mobilized apheresis product. Anticoagulant Citrate Dextrose Solution, USP Formula A (ACD-A) can be used if anticoagulants are specified in the apheresis protocol. Do not use other anticoagulants for collection of PBPC that will be processed with this device.
2. Remove an aliquot of the cell product and determine the number of nucleated white blood cells and hematocrit.
3. Centrifuge the product and remove the supernatant plasma. Resuspend the pellet in a total volume of 250 mL using 1X Dulbecco's Phosphate Buffered Saline (D-PBS) (Ca^{2+} Mg^{2+} free) or 0.9% Sodium Chloride Injection USP (Ca^{2+} Mg^{2+} free). The maximum number of nucleated cells per device is 5×10^{10} cells (2×10^{8} cells/mL). *Ensure that the hematocrit of the cell suspension mixture is no more than 20%.*

Device Preparation:

- Prior to filling the Separation Container, assemble the device as described in this section and as shown in Figure 2.
- Perform all device assembly and cell manipulations under aseptic conditions.

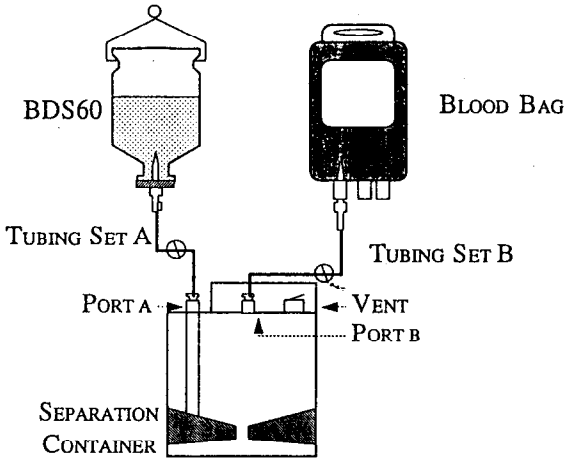


Figure 2 Device Assembly

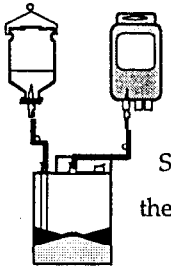
1. *Shake the bottle of BDS60 to mix the contents well.* As water droplets may condense on the sides of the bottle, this step is important to ensure the proper density of BDS60 solution. Any foaming or bubbles that form should dissipate quickly. Remove the flip tear cap from the bottle. **Do not remove the metal outer ring.** Swab the rubber stopper with alcohol.
2. *Open the Tubing Set A pouch.* Close the blue tubing clamp. Remove and discard the Tubing Set A spike cap. Insert the vented spike into the rubber stopper of the bottle. Open the spike air vent.
3. *Open blue Port A on the Separation Container and* **save the blue cap.** Remove and discard the second cap of Tubing Set A. Connect Tubing Set A to Port A. Invert the bottle and hang by the inner ring tabs in the plastic base cap.

4. *Open Tubing Set B pouch.* Close the red clamp. Remove and discard the Tubing Set B spike cap. Insert the Tubing Set B spike into the leukapheresis bag.

5. *Open Port B on the Separation Container and* **save the red cap.** Remove and discard the cap of Tubing Set B. Connect Tubing Set B to Port B. Hang the leukapheresis bag. **Open the Separation Container air vent.**

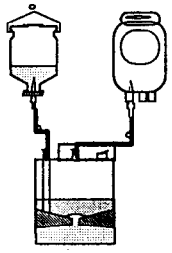
Protocol:

1. *Fill the lower chamber with BDS60:* Release the blue clamp of Tubing Set A and fill the lower chamber of the Separation Container with BDS60. **Fill to just above the top of the insert orifice.** Small bubbles may emerge into the upper chamber as the lower chamber becomes completely filled. When the lower chamber is filled, close the blue clamp of Tubing Set A to prevent further filling of BDS60 into the upper chamber of the Separation Container.

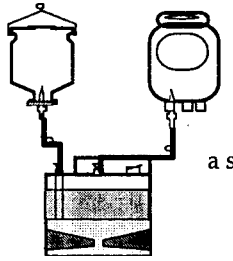


Note: After the lower chamber is filled, a single bubble may remain in that chamber; this bubble is expected and will not interfere with device performance. Ensure the Separation Container is level during filling, otherwise excessive amounts of air may remain in the lower chamber.

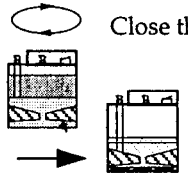
2. *Fill the upper chamber with sample:* Gently tilt the device so that the base of the air vent side is lifted off the surface slightly (~one cm). Release the red clamp of Tubing Set B. Drain the 250 mL cell suspension into the upper compartment of the Separation Container. After the first few mLs of sample have been transferred, the device may be put level on the surface. Do not use pressure to load samples as this may result in mixing with BDS60 in the lower chamber.



3. *Resume underlaying with BDS60:* After the Separation Container has been charged with sample, release the blue clamp of Tubing Set A and finish filling the Separation Container until the BDS60 bottle is empty. The BDS60 will underlay the product, resulting in a sharp interface.



4. *Perform the density separation:* Disconnect and discard Tubing Sets A and B. Close the Separation Container air vent. Close ports A & B with their respective blue and red caps. Centrifuge the Separation Container at 850g for 30 minutes at ambient temperature in the range of 20° - 25°C.



Harvest and Wash the Cells:

Note: Upon removal of the container from the centrifuge, observe the interface in the upper chamber of the Separation Container prior to opening the air vent. A successful cell separation is indicated by the presence of a BDS60/cell interface that may appear as a cell cloud or band. If a cell cloud or band is not visible, the cell separation procedure may have failed. In this event, harvest the contents of the upper chamber into a sterile cell transfer bag as described below. Next, collect the cells from the lower chamber following the procedure entitled "Optional Cell Recovery from Lower Separation Chamber" on page 8. Wash the cells from both chambers separately as described below and then determine the number of CD34+ cells recovered in each chamber. A sufficient number of cells should be cryopreserved (>2 x 10^6 CD34+ cells/kg) to ensure rapid engraftment. In the event of a performance failure, these cells may be obtained from the upper chamber, the lower chamber, or by combining the contents of the upper and lower chamber. **Reprocessing of cells of the DACS SC is not advised.**

1. *Prepare device for cell recovery.* **Under Aseptic conditions open the Separation Container air vent upon removal from the centrifuge.** Open the Tubing Set C Pouch and close the tubing clamp. Connect Tubing Set C to an empty 600 mL cell transfer bag. Tubing Set C possesses a luer lock that will allow it to attach to cell transfer bags directly or through a sterile connector.
2. *Harvest cells from the upper chamber of the device.* Invert the Separation Container. Open the clamp of the Tubing Set and allow cells to drain from the upper chamber of the Separation Container into a sterile cell transfer bag.
3. Clamp the Tubing Set and disconnect it from the Separation container and discard the container.
4. *Wash the cells using a cell transfer bag.* Connect the Tubing Set to a bag with wash solution. Open the Tubing Set clamp and fill the bag containing cells completely with wash solution.
5. Clamp the Tubing Set and disconnect it from the wash solution bag. Cap the Tubing Set.
6. Place the bag with cells in a centrifuge and coil and position the Tubing Set in a manner that it does not interfere with the centrifugation process.
7. Pellet the cells by centrifugation at 850 g for 10 minutes at room temperature (20°–25°C) with the brake off. Ensure that the centrifuge is well balanced.
8. Remove the bag from the centrifuge. *Use caution not to mix up the cells in the pellet.* Connect the Tubing Set to an empty transfer bag equipped with a compatible docking port. Open the clamp and express as much supernatant as possible without removing cells from the pellet.
9. Disconnect the Tubing Set and discard the supernatant. Clamp and cap the Tubing Set and resuspend the cells in Wash Solution (0.9% saline, or Ca^{2+} Mg^{2+} free D-PBS) to 600 mL. Wash the cells twice by two cycles of centrifugation and resuspension. Resuspend the cells in approximately 600 mL total volume.
   Note: It is important to perform the cell washing steps as described to ensure adequate removal of BDS60 solution from the cell preparation.
10. *Cell resuspension.* After the second wash, remove the supernatant and resuspend the cells in the appropriate media for subsequent processing or storage.

Optional Cell Recovery From Lower Separation Chamber:

Note: The procedure described below is provided in the event of device/protocol problems.

1. Connect a 60 cc syringe to Port A of the Separation Container.
2. Tilt the Separation Container and withdraw the wash solution with the 60 mL syringe, mixing the cells several times to dislodge the pellet. Cells may also be further dislodged, if necessary, by using the syringe to gently transfer cells and wash solution in and out of container.
3. Transfer cells to a cell transfer bag and wash as described above.

STORAGE CONDITIONS:

Upright at room temperature (15°-30°C)

Troubleshooting:

If you have a problem performing the procedure, the following suggestions may be helpful to you. If you need additional help, please call Fresenius at 800-909-3872.

Problem	Reason / Suggestion
DACS™SC packaging arrives opened or damaged	Do not use. Contact Fresenius.
After centrifugation, solution squirts out from the container when the ports are opened.	After centrifugation, open the air vent(s) prior to opening port(s).
Fluid won't drain through tubing	Check if air vent(s) is open. Check if clamp is open. Possible airlock in tubing filtered air vent; try tapping on the side of the spike. Increase the height distance between the two tubing set ends, e.g., by lowering one container. Container air vent filter may be too wet; allow it to dry out.
Red blood cells are present in the upper chamber of the Separation Container after centrifugation.	Ensure that hematocrit is < 20% in the apheresis product. Ensure adequate centrifugation time and speed.
High total nucleated cell recovery	Ensure that sample was < 4 hours old prior to separation.
Low $CD34^+$ cell recovery	Ensure that BDS60 was at RT (20°-25°C) prior to separation. Ensure adequate mixing of BDS60 prior to use.
Device leakage	Ensure that the upper chamber was not overfilled.
Clumped cells	Ensure that: Plasma was expressed during sample preparation. Only Ca^{2+} Mg^{2+} free solutions were used. The total nucleated cell count does not exceed $5x10^{10}$ cells.

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ORDERING INFORMATION

DACS™SC: *Part No. 9091001*

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