TECHNICAL MANUAL
VERSION 2.4.0

RoboSep™

The Fully Automated Cell Separator

STEMCELL™ TECHNOLOGIES

SAFETY INFORMATION AND WARNINGS

This instrument is intended for research use only and must be operated by trained professionals.

To ensure adequate cooling of RoboSep™, place it on a clean, flat surface, and be sure that there is at least 10 cm (4 in) of clearance around each side of the instrument. Make sure that the bench space is free of any foreign objects or debris that could block the fan vents.

Always connect the power supply to a 3-prong, grounded AC outlet rated 4A, 110–240 V using the AC power cord provided with RoboSep™. Do not use an adapter to a two-terminal outlet. Before plugging the instrument in, be sure that the correct fuses are installed. Use two Type F 250V 4A 5X20mm fuses.

Electrical devices pose the risk of electric shock. To reduce the risk of shock, do not open any covers that are fastened with screws. While RoboSep™ is designed to withstand spills on its exterior surface, do not allow fluids to enter the interior of the instrument. In the event of such a spill, disconnect the power cable before cleaning up.

Do not operate RoboSep™ in extreme humidity or in conditions that can create condensation. Protect the instrument against dust and moisture, and avoid physical shock and strong forces. Do not exert excess pressure on the screen.

The EasySep™ magnets on the RoboSep™ carousel generate strong magnetic fields. Keep away from pacemakers, watches and other objects that respond to magnetic fields.

As RoboSep™ weighs 32.6 kg (72 lb), exercise caution when moving the instrument. It is recommended that two people are present to lift or move the instrument safely.

In case of malfunction, call STEMCELL Technologies Inc for service. Contact information is located at the back of this manual. Do not attempt to fix the instrument yourself. Never remove the outer casing of RoboSep™. There are no user-serviceable parts inside the instrument.

NOTICE

RoboSep™ is designed and certified to meet UL-CAN/CSA-CENELEC EN 61010-1 safety requirements. Safety certified products are safe to use when operated in accordance with the technical manual. This instrument should not be modified in any way. Alteration of this instrument will:

- Void the manufacturer's warranty.
- Void the 61010-1 safety certification.
- Create a potential safety hazard.

This product conforms to the "Class A" standards for electromagnetic emissions intended for laboratory equipment applications, according to FCC (Part 15) and CENELEC EN 61326. It is possible that emission from this product may interfere with some sensitive appliances when placed nearby or in the same circuit as these appliances. The user should be aware of this potential, and take appropriate measures to avoid interference.

STEMCELL Technologies is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument or software not performed by STEMCELL Technologies or an authorized agent.

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1 INTRODUCTION

RoboSep™ (Catalog #20000) is a laboratory instrument that automates EasySep™ immunomagnetic cell separation. EasySep™ is a column-free magnetic cell separation technology that enables both positive and negative selection of desired cells from virtually any species. Using an integrated color touch screen, RoboSep™ can be programmed to purify cells from up to four samples at once. The user simply loads the consumables as indicated on the built-in touchscreen and starts the experiment; RoboSep™ then automatically executes the EasySep™ immunomagnetic labeling and magnetic separation steps using disposable pipette tips.

RoboSep™ is intended for research applications only. Please make sure that you have read page ii of this manual before using the instrument.

1.1 SYSTEM DESCRIPTION

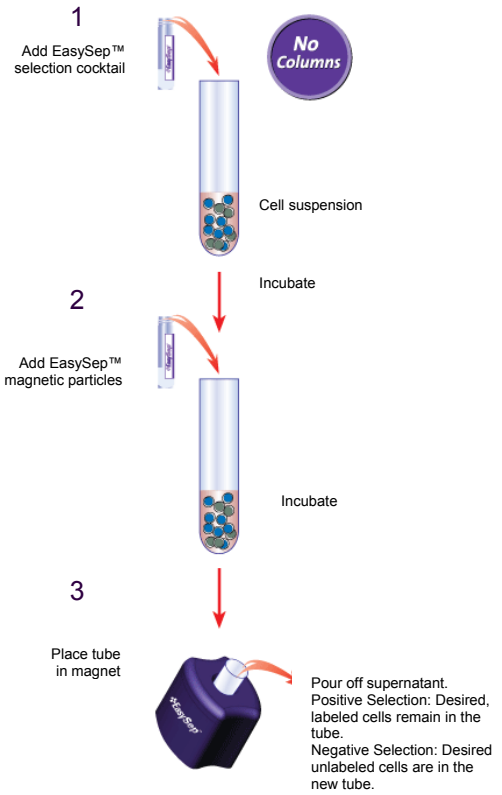
1.1.1 EasySep™ Magnetic Cell Separation Technology

EasySep™ is a powerful immunomagnetic cell selection procedure that combines the specificity of monoclonal antibodies with the simplicity of a column-free magnetic system. EasySep™ can be used as either a positive or a negative selection approach to purify your cells of interest.

With **EasySep™ positive selection**, highly purified cells are obtained by targeting the cells of interest with antibodies directed against one or more specific cell surface antigens. These targeted cells are cross-linked to EasySep™ magnetic particles. The sample tube containing the cells is then placed directly in a specially designed EasySep™ magnet. The unique magnet generates a high gradient magnetic field in the interior cavity that is strong enough to separate cells labeled with EasySep™ magnetic particles without the additional magnetic field gradients provided by a column matrix. The cells that are not bound to the magnetic particles are removed either by pipetting off (RoboSep™) or by inversion of the magnet (manual EasySep™). To obtain a highly purified cell population, the cells remaining in the tube can undergo additional separations prior to being collected by removing the tube from the magnet (Figure 1). Unlike larger particles used with other column-free systems, the EasySep™ magnetic particles used in positive selection procedures do not interfere with subsequent flow cytometric analysis and do not need to be removed.

For **EasySep™ negative selection** applications, unwanted cells are targeted for depletion using a cocktail of monoclonal antibodies directed against specific cell surface antigens. The labeled unwanted cells are then cross-linked to EasySep™ magnetic particles. The sample tube containing the cells is placed in the specially designed EasySep™ magnet. The desired (unlabeled) cells are removed either by pipetting off (RoboSep™) or by inversion of the magnet (manual EasySep™).

Figure 1- RoboSep™ fully automates the EasySep™ procedure for positive (illustrated below) or negative selection.

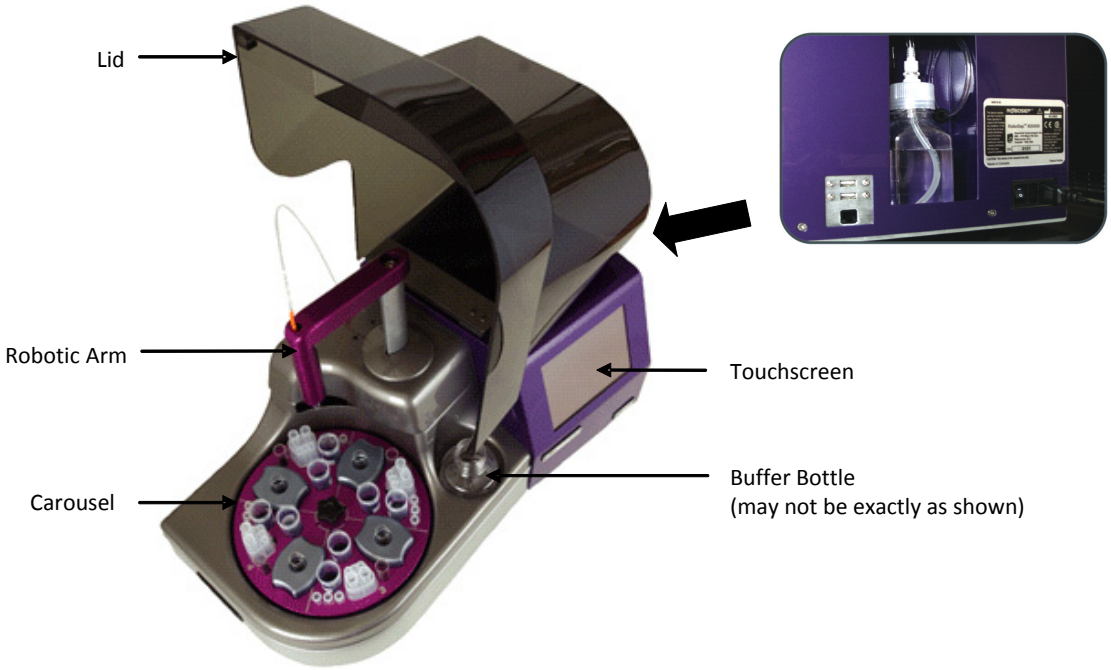


1.1.2 Robotic System Overview

RoboSep™ is a pipetting robot comprised of four main mechanical components that are operated under computer control (Figure 2):

- The Robotic Arm
- The Carousel
- The Tip Stripping Arm (not visible in Figure 2)
- The Hydraulic System

Figure 2 - RoboSep™ Instrument



With these systems acting in concert, RoboSep™ performs the following actions to automate EasySep™:

- Accurately dispenses magnetic labeling reagents into the cell samples using disposable 1 mL tips
- Mixes and transfers the sample between tubes using a 5 mL tip
- Transfers buffer from the buffer bottle to tubes on the carousel using a second 5 mL tip
- Keeps track of the appropriate incubation times

Scheduling software sets the sequence in which RoboSep™ will execute all of the actions required to process up to 4 samples. This gives RoboSep™ the flexibility to perform different types of separations at the same time. A custom-designed single-use tip rack contains all the pipette tips necessary to process a single sample (Figure 3). Each tip has its own compartment to prevent cross-contamination and the rack is sterilized by gamma-irradiation.
NOTE: Remove the lid of the tip rack prior to use.

Figure 3 - RoboSep™ filter tip rack

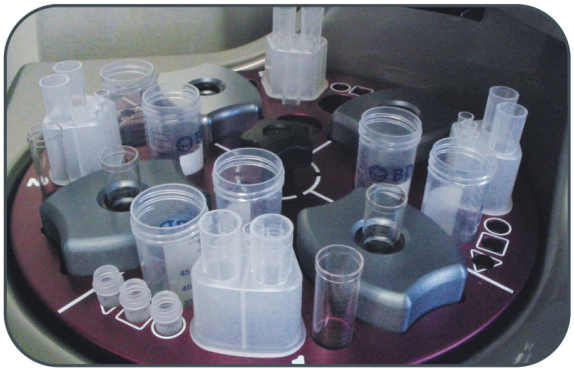
(a) With lid (b) Without lid

1.1.3 The Robotic Arm and Carousel

The robotic arm (Figure 4) is driven by two independent stepper motors to provide rotation and vertical movement. Rotation of the carousel is driven by a single stepper motor. The carousel is divided into 4 quadrants (Figure 5); a quadrant defines the minimum functional area for processing a sample. Each quadrant is designed to accommodate a 14 mL sample tube, a magnet with a 14 mL separation tube, up to 3 reagent vials, a tip rack and 2 multifunctional 50 mL tubes.

Figure 4 – The robotic arm is capable of vertical and rotational motion. The stainless steel tip head at the end of the arm is used to pick up pipette tips.

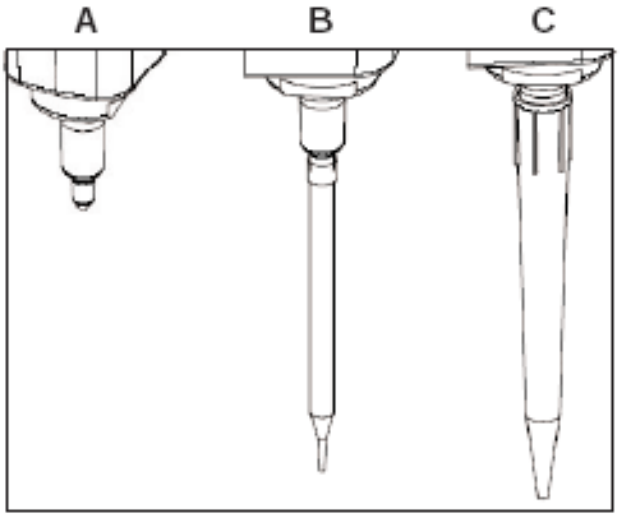
Figure 5 – The carousel is divided into quadrants as pictured below. This figure shows each quadrant fully loaded with a magnet, tip rack, reagent vials, and tubes required to carry out a separation.



By coordinating the rotational and vertical movement of the arm with the rotation of the carousel, RoboSep™ picks up disposable tips from the custom tip racks on the carousel and then uses them to transfer fluids from one container to another or to mix the fluids in a given tube. Fluids are dispensed into the sample liquid to ensure aerosol containment.

The robotic arm can pick up both 1 mL and 5 mL disposable pipette tips using a unique tip head that has two conical tip sealing surfaces (Figure 6). This dual tip capability enables RoboSep™ to accurately dispense volumes from 12.5 µL up to 10 mL. RoboSep™ uses 1 mL tips to aspirate and dispense the magnetic labeling reagents and 5 mL tips to transport the sample and buffers.

Figure 6 – The tip head (label A) has two conical surfaces that enable it to engage with the 1 mL (B) and 5 mL (C) disposable pipette tips.



1.1.4 The Tip Stripping Arm

RoboSep™ disposable tips are returned to the tip rack after each use. This eliminates the need for a waste tip area on the instrument and facilitates easy clean-up after a run by allowing the entire tip box to be disposed of appropriately. Figure 7 illustrates the automated pipette tip removal process.

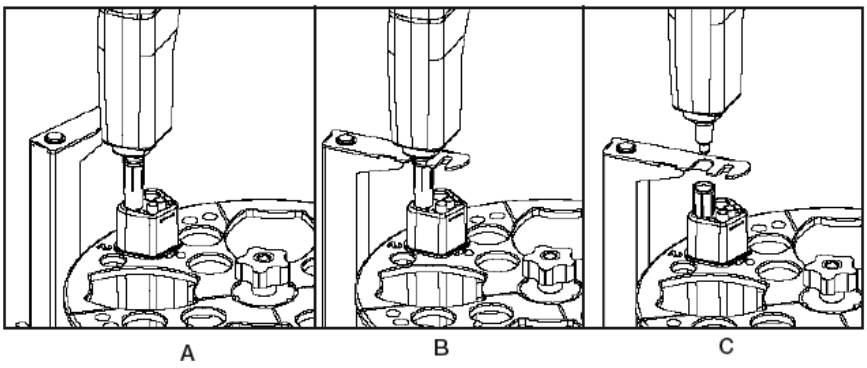


Figure 7 – Disposable 5 mL pipette tip being replaced in the tip rack. The robotic arm places the used tip part way into the rack (A). The tip stripping arm swings out to engage the tip head just above the tip (B). The robotic arm then moves up and the tip falls into the tip rack (C).

1.1.5 The Hydraulic System

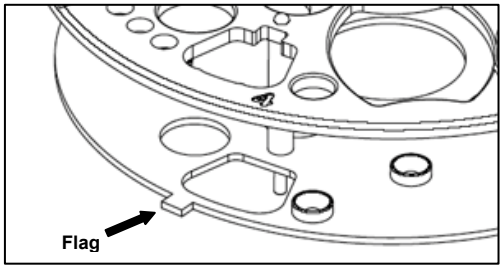
The hydraulic system uses sterile deionized water driven by a positive displacement piston pump. The pipette handling tip head is connected to the reservoir bottle containing the sterile deionized water via the pump. The only visible parts of this system are the water bottle and the tubing running from the console to the robotic arm. The hydraulic fluid does not make contact with the user's samples or reagents, and because it is deionized water, it is less damaging to the piston seal than salt solutions or buffers. The RoboSep™ pump therefore has a substantially longer operating life than the seals on syringe pumps used in other instruments and does not require any user maintenance. The use of water as a hydraulic fluid instead of air allows for accurate high-speed dispensing: the water effectively acts as an incompressible extension of the pump piston, allowing the system to operate with a dead air volume similar to that of a manual pipettor.

1.1.6 Homing the Carousel and Robotic Arm

RoboSep™ tracks the position of the robotic arm and carousel relative to physical markers called homing flags. A home position is registered by the computer when a homing flag trips an optical sensor. The carousel homing flag is readily visible as a projection on the bottom plate (Figure 8). This projection trips an optical sensor which can be seen on the front inside wall of the bowl when the carousel is removed. The robotic arm homing flags and sensors are not visible. Homing of the carousel and robotic arm is performed automatically at start-up, at the beginning of each cell separation experiment and after the instrument has been paused during a run. The robotic arm home positions are detected again periodically throughout a run.

To ensure correct operation of the instrument, it is essential that no mobile parts be moved or touched during an experiment. Homing is the only mechanism by which RoboSep™ determines the location of items on the carousel; any disruption of the carousel or the arm during operation can cause pipetting actions to fail. In order to prevent such disruptions, RoboSep™ operates with a transparent lid over the arm and carousel. If the lid is opened during an experiment, the instrument will pause once the current action is complete. Once the lid is closed, RoboSep™ will automatically execute a homing sequence and resume the experiment.

Figure 8 – The carousel home flag is visible as a projection from the bottom plate in this schematic diagram



1.2 INSTRUMENT SET-UP

1.2.1 Unpacking the Instrument

A STEMCELL Technologies authorized agent will be present to help you unpack and install the instrument. Please note that proper operation of RoboSep™ requires the correct installation of the instrument.

1.2.2 Instrument Placement

To ensure adequate cooling of RoboSep™, the instrument should be placed on a clean flat surface with at least a 10 cm (4 in) clearance around each side. Make sure that the bench space is free of any foreign objects or debris that could block the instrument's fan vents.

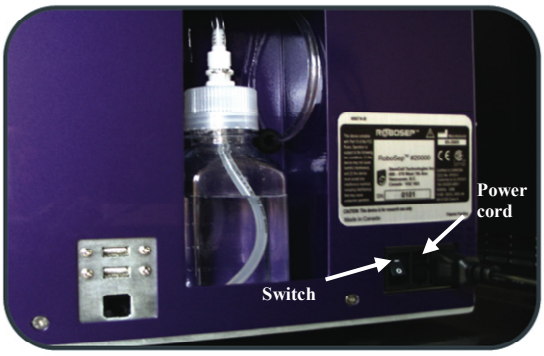
RoboSep™ is designed to fit in most biocontainment hoods for sterile operation; it is not specified for use in a cold room or refrigerator (2 - 8°C, 39°F) (see Section 7 – Specifications). STEMCELL Technologies recommends operating RoboSep™ with the instrument's lid in place to prevent accidentally interfering with the operation of the robotic arm. Talk to your STEMCELL Technologies authorized agent if you wish to remove the lid from RoboSep™ for installation in a biocontainment hood.

1.2.3 Power and Data Connections

Plug in the power cord provided with RoboSep™ to the power supply outlet located at the bottom right side of the instrument, next to the fuse box and the power switch (Figure 9), and connect to a 3-prong, grounded AC outlet rated 4A, 110–240 V. Do not use an adapter to a two-terminal outlet.

Data connections to RoboSep™ are through an Ethernet port and 2 USB ports. Section 4 – Communications and File Transfer provides more detailed instructions on setting up RoboSep™ on a network.

Figure 9 – Side view of RoboSep™ showing power and data connections, as well as the hydraulic fluid bottle and power switch.



1.2.4 Powering Up and Shutting Down

Power up RoboSep™ by activating the power switch located at the bottom right side of the instrument (Figure 9). RoboSep™ first loads the computer operating system and then the instrument control software and user interface. The touchscreen provides visual confirmation that these processes are occurring. After a short period of time, the robotic arm and carousel will move. The start-up sequence is complete once the start screen is visible (see Figure 10 on page 7 for a screenshot).

Note: The start-up sequence may take longer than usual if a USB device is attached to RoboSep™.

The procedure to shut down RoboSep™ is as follows:

1. Navigate to the "Instrument Tasks" section of the graphical user interface by pressing the appropriate tab at the top of the touchscreen.
2. Under the "Maintenance" tab, press "Basic Shutdown Protocol."
3. Wait until the screen becomes black. Once it has done so, turn off the instrument's power switch.

1.2.5 Priming the System

RoboSep™ performs a priming sequence at the beginning of every cell separation protocol to minimize the effect of air bubbles that naturally accumulate in the hydraulic system. However, when RoboSep™ is used for the first time, or if the hydraulic fluid bottle runs empty, it is necessary to run a separate priming protocol by following the steps below:

1. Fill the hydraulic fluid bottle with 250 mL of sterile deionized water and connect the bottle to the hydraulic system tubing in the compartment located at the right of the instrument (Figure 9).
2. If the instrument is turned off, switch it on and wait until the end of the start-up sequence as described in section 1.2.4.
3. Press the “Instrument Tasks” tab, select “Basic Prime” under the “Maintenance” tab and then press the green “Load” button.
4. Place a 50 mL tube in the appropriate slot of the carousel as indicated on the screen.
5. Press “Run.”

If no water is expelled from the tip head into the waste tube by the end of the priming sequence, there could be a problem with the connections at the hydraulic fluid bottle (see Section 6 – Troubleshooting).

1.3 RUNNING ROBOSEP™: QUICK START INSTRUCTIONS

Note: Before using RoboSep™, ensure that the hydraulic fluid bottle is full (250 mL of sterile deionized water).

The following is a brief quick start guide to using the RoboSep™ fully automated cell separator; detailed instructions on running RoboSep™ are provided in Section 2 of this manual. To quickly execute a run on RoboSep™:

1. Power up RoboSep™ and wait for initialization to complete.
2. Follow the instructions on the message bar at the top of the screen. Press “Select Protocol” to bring up the list of available protocols (see Section 3.1.2 to customize the protocol list). Choose the desired protocol from the list and then enter the sample volume. Repeat this process until all the desired protocols (up to four) are selected.
3. Press “Load” to begin the loading sequence. Lift the lid and load the indicated carousel quadrant(s) with the items listed on the left of the touchscreen. Press “Loaded” once each quadrant is ready. When all quadrants are loaded, close the lid.
4. Press “Run” and confirm you are ready for the instrument to start. RoboSep™ will now complete the selected protocols unless it is paused or halted. The time remaining until completion is displayed on the screen.
5. Once the run is complete, press “Unload,” open the lid, and collect the cells of interest. For positive selection, the cells of interest are in the magnet tube. For negative selection, the cells of interest are in the 14 mL sample tube of the second quadrant (if using 2 quadrant negative selection), or the 50 mL negative fraction tube of the first quadrant (if using 1 quadrant negative selection).
6. Unload the rest of the carousel and store the reagents appropriately. Discard the tip boxes containing the used tips in the appropriate waste container.

The instrument is now ready for another run. If no more runs are planned for the day, or if a spill occurred, clean the instrument as detailed in Section 5 – Care and Maintenance. To conserve energy, turn off the instrument at the end of the work day.

Note: RoboSep™ protocols may differ from the standard manual separation protocols because they have been optimized for an automated system. Unless otherwise noted, all RoboSep™ procedures have been optimized for use at room temperature (15 - 25°C), regardless of the temperature recommended for the manual procedure.

2 RUNNING ROBOSEP™: DETAILED INSTRUCTIONS

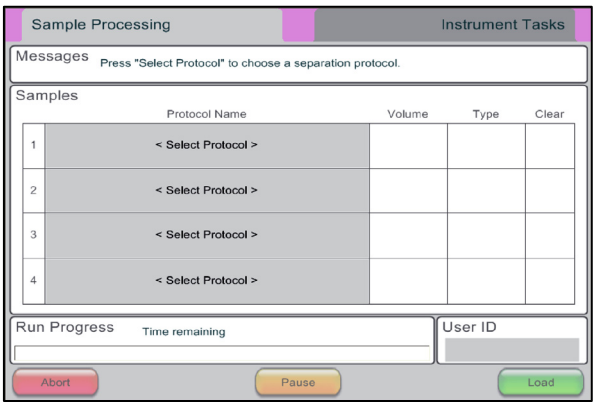
2.1 INTRODUCTION

When the RoboSep™ cell separator is powered up (see power-up instructions in section 1.2.4), the RoboSep™ graphical user interface loads automatically; upon completion of start-up, the "Sample Processing" start screen appears.

Note: Please wait for the end of the start-up sequence before touching the touchscreen, the lid, or other moving parts of the instrument, so as to not interfere with the start-up process.

The user interface consists of two main sections, defined by tabs at the top of the screen: the "Sample Processing" section, which is shown at start-up, and the "Instrument Tasks" section (Figure 10). The light grey tab indicates which section is currently active. You can switch from one section to the other at any time by touching the appropriate tab.

Figure 10 – Start screen of the RoboSep™ user interface



2.2 SETTING UP A RUN

Navigate to the "Sample Processing" section of the user interface (Figure 10) by touching the appropriate tab at the top of the RoboSep™ user interface. The sample processing screen is initially divided into five areas: Messages, Samples, Run Progress, User ID, and a row of action buttons along the bottom. The areas' functions are:

- The Messages area indicates what step is next in the set-up process to start running RoboSep™.
- The Samples area is divided into four rows corresponding to the four quadrants on the carousel. Each row has columns to display the protocol name, the sample volume (in mL), and the type of cell selection protocol. Once protocols have been selected, this area indicates on which quadrant of the carousel each protocol will be run.
- The Run Progress area has a progress bar and countdown clock that shows the time to completion of the current run.
- The User ID area allows the user to enter his/her unique code for reporting purposes.
- The "Abort," "Pause," and "Load" action buttons allow the user to issue the appropriate commands to terminate, pause, or start a run.

2.2.1 Entering a User ID

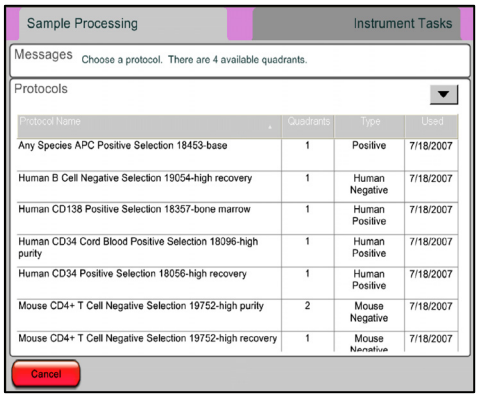
A User ID can be entered at any point before a run begins; it is stored internally and is included in the report file that is generated after each run (see Section 2.4). User IDs are useful for keeping track of usage in a multi-user environment. To enter a User ID:

1. Press the grey box under the "User ID" heading. A numeric pad will pop up (note: a keyboard can also be used).
2. Key in the user ID, and press the "Enter" key.

2.2.2 Selecting Protocols

RoboSep™ is delivered with a set of standard protocols optimized for use with "typical" samples (e.g. peripheral blood mononuclear cells from normal donors). Standard protocol names match the corresponding EasySep™ product name and catalog number. Certain protocols also include a descriptor (e.g. "high recovery") when more than one protocol is provided for the same product. If working with atypical samples (e.g. clinical/patient samples), protocols can be modified as appropriate and given a different descriptor (see Section 3 – Customizing RoboSep).

Figure 11 – List of protocols to select from



The RoboSep™ protocol selection procedure is described below.

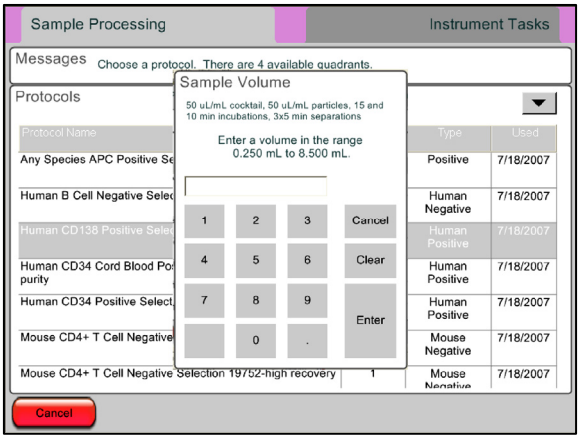
1. Choose a protocol to run:
   a. Touch "Select Protocol" in any row of the Samples area (Figure 10). A list of the available protocols appears on the screen (Figure 11).
   b. Select a protocol by touching the protocol name. A numeric pad pops up (Figure 12).

c. Key in the sample volume in mL, and press the "Enter" key. The original "Sample Processing" screen will re-appear with the selected protocol in the appropriate quadrant row.

Note: A short description of the protocol appears in the Samples area.

2. Review/edit the information:
   a. To remove the protocol, press the "X" in the last column.
   b. To change the protocol, press the protocol name and repeat the protocol selection process above.
   c. To change the sample volume while keeping the same protocol selected, press the sample volume for this protocol, and enter a new volume (Figure 12).
3. Repeat the protocol selection process until all the desired protocols are entered.

Figure 12 – Entering sample volumes



Up to four protocols can be chosen (they can be the same or different). When a negative selection protocol with two rounds of separation is selected, the interface automatically assigns two adjacent quadrants for the protocol. Once three quadrants are occupied, only protocols that use one quadrant will be displayed.

Protocol Order

The protocols are listed in order of last use, with the most recently used protocols at the top of the list (Figure 11). The protocols can also be sorted by name or type. To change the basis of sorting, touch the appropriate column heading. For example, to sort protocols in alphabetical order press the Protocol Name heading. An arrow in the heading indicates if the list is sorted in ascending or descending order; touch the column heading again to toggle between these two options. The user can move up and down the list by using the arrows at the top right corner of the protocols window.

2.2.3 Preparing Cells

Refer to the Product Information Sheets that come with your EasySep™ reagents and/or magnet for instructions on how to prepare cells for separation using RoboSep™. It is important to use the recommended cell concentration for best results. Ideally the sample should be a single cell suspension. However, RoboSep™ tips will not clog if small aggregates are present.

2.2.4 Reagents and Resources

The sample tube and separation tube are 14 mL (17 x 100 mm) round-bottom tubes. We recommend Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057). The waste tube and negative fraction tube/lysis buffer tube are 50 mL conical bottom tubes (e.g. BD Biosciences, Catalog #352070). The Service Rack (Catalog #20101) is provided to hold the reagents and sample prior to loading them on the carousel and to keep track of the caps during a run (Figure 13). Like the carousel, the rack is laid out in quadrants, with symbols on the reagent storage locations to assist with keeping tubes together with the correct caps (▲ corresponds to the Magnetic Particles vial; ■ corresponds to Selection Cocktail vial; ● corresponds to Primary Antibody vial).

Figure 13 – The Service Rack is divided into quadrants and features symbols on the reagent storage locations to assist keeping tubes together with the correct caps.

2.2.5 Loading the Carousel

Once all the desired protocols have been selected and the cell samples have been prepared, the carousel can be loaded:

1. Press the "Load" button. If the same protocol has been selected in more than one quadrant, a pop-up window will open to give you the option of using a single set of reagents for all identical separations (Figure 14). Alternatively, one set of reagents may be used for each individual separation.
2. An image of the carousel will appear (Figure 15). The active quadrant number(s) is displayed at the bottom right of the window. At the top center of the window, the name of the protocol assigned to this quadrant is also displayed. On the left hand side of the window is a list of all the reagents and resources that need to be loaded for the current separation (see Section 2.2.4 for suitable tubes). The list starts with items near the center of the carousel and ends with items near the rim to facilitate aseptic loading. The user can move up and down the list by pressing the arrows located above and below.
3. Touch the first item on the list. The item will be highlighted in green and its location on the carousel indicated by a green circle. Turn the carousel so that the indicated quadrant is in a convenient position and place the item in the indicated location. Continue down the list, loading each item in turn.

Figure 14 – One set of reagents can be used for multiple identical separations

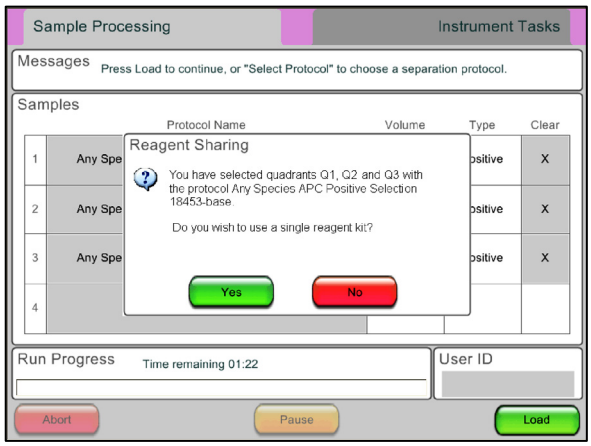
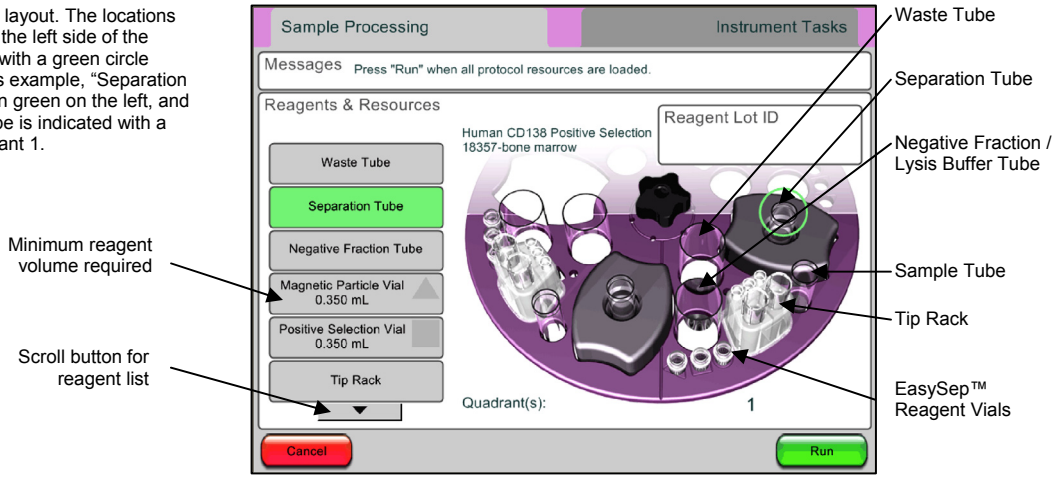


Figure 15 – Carousel layout. The locations of the items listed on the left side of the screen are indicated with a green circle when selected. In this example, "Separation Tube" is highlighted in green on the left, and the location of the tube is indicated with a green circle in Quadrant 1.



4. Load reagents. The minimum reagent volume required for a given cell separation procedure is displayed below the name of each reagent. Verify that your vial has the minimum required volume. To assist in proper placement, the reagent vial labels have symbols that correspond to symbols on the carousel and on the touchscreen. For recording reagent lot numbers and sample IDs, first select the item on the list and then select the grey box under the "Reagent Lot ID" heading. Alternatively, STEMCELL offers a barcode reader (Catalog #20501) that can scan barcodes on reagent vials and sample tubes to record reagent lot # and sample IDs (see Section 2.2.6).

Important Notes:

- *RoboSep™ uses EasySep™ reagents in 1.5 mL vials from Sarstedt. Use of any other vial will cause RoboSep™ separations to fail.*
- *Certain negative selection protocols use D magnetic particles that can be pulled to the side of the sample tube by the external magnetic field of "The Big Easy" EasySep™ Magnet. While this does not affect performance, it can be prevented by inserting a magnet shield between the magnet and the sample tube.*

- *When loading the tip rack, make sure that the cap has been removed (see Figure 4). Be careful not to open the lower joint when removing the cap from the tip rack. The rack must be fully seated so that the tips are in the correct location for pick-up by the robotic arm.*
- *The cap must be removed from the RoboSep™ Buffer Bottle (Catalog #20104) and ensure there is sufficient volume, as indicated on the screen.*

5. Press the green "Loaded" button when all the reagents and resources are loaded on the active quadrant(s). The reagents and resources required for the next selected protocol will appear.
6. Repeat steps 2 – 4 until all the required quadrants are loaded. Close the instrument lid. The instrument is now ready to run a separation.

2.2.6 Entering Reagent Lot ID with a Barcode Scanner

Lot numbers on RoboSep™ reagents can be scanned and automatically recognized by a barcode scanner (Catalog #20501). The scanned information will record the lot number in the "Reagent Lot ID" field shown on the right hand corner of Figure 15. All recorded information can be retrieved from the RoboSep™ Log Files (see Section 2.4 Report Files) for convenient tracking of RoboSep™ reagents.

To use the barcode scanner, connect the scanner to the RoboSep™ unit via the USB outlet. Wait for a few seconds for the scanner to connect to the unit. A red scanning laser should appear when the scanner is triggered. Upon loading the carousel and recording the lot number, align the scanner and scan the barcode on the RoboSep™ reagent vial to record the lot number. The lot number should appear on the "Reagent Lot ID" field.

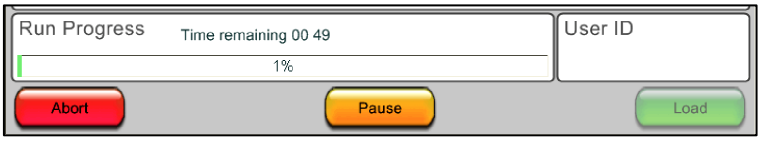
2.2.7 Running the Separation

After the instrument has been readied for separation, the following steps will start the experiment:

1. Press the green "Run" button (Figure 15) to start the cell separation experiment. A warning message will appear asking the user to verify that all the reagents and resources are correctly loaded and that nothing can interfere with the movements of the instrument. **Please ensure that the lids have been removed from all vials and resources, including the buffer bottle.**
2. Once everything is verified to be clear, press "Confirm" (Figure 16). Observe that the initial prime step ejects water from the tip head into the waste tube in carousel Quadrant 1 (Q1). This ensures that the hydraulic fluid system is properly connected.

Figure 16 – Warning message

Figure 17 – Run Progress. Once a run has begun, progress is shown at the bottom left, with an indication of how much time remains.

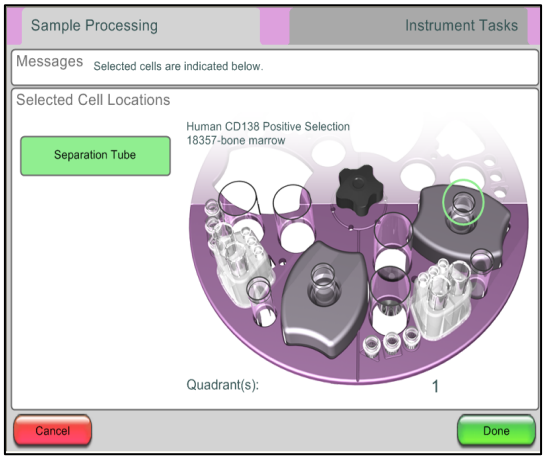


The experiment is now in progress. The time remaining until completion is displayed at the bottom of the screen (Figure 17). The machine will beep when all separations are complete.

2.2.8 Collecting Cells

To collect the cells of interest, press "Unload" and allow 5 seconds for the robotic arm and carousel to move to a safe position before opening the instrument lid. The position of the isolated target cells will be indicated on the RoboSep™ touchscreen (Figure 18).

Figure 18 – Following separation, the location of the desired cells is indicated by a green circle.



For positive selection:

- Remove the 14 mL tube containing the isolated cells from the magnet. Promptly re-suspend the selected cells in an appropriate volume of buffer.
- If desired, collect the 50 mL tube containing the depleted fraction located to the left of the tip box.

For negative selection:

- If two rounds of separation were performed, collect the enriched cells in the 14 mL tube located to the left of the magnet on the second quadrant used for the negative selection procedure.
- If only one round of separation was performed, collect the 50 mL tube containing the enriched cells located to the left of the tip box.
- If desired, collect the 14 mL tube(s) containing the labeled cells located in the magnet(s).

Once the separated cells have been collected:

- Unload the carousel and store the reagents appropriately.
- Discard the tip boxes containing the used tips in the appropriate waste container.
- If prompted, refill the hydraulic fluid bottle with sterile deionized water for further experiments.

The instrument is now ready for another experiment.

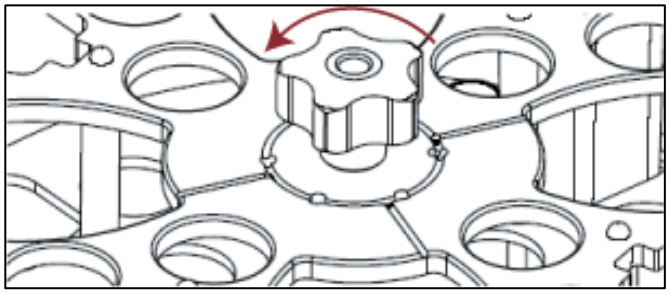
2.2.9 Removing the Carousel

The carousel can be easily removed from the instrument for cleaning or to facilitate loading and unloading of the samples and reagents. Some users may prefer to remove the magnets prior to removing the carousel to reduce its weight. Position the carousel with Quadrant 1 on the front side of the machine (facing the user) before removing it so that the optical sensor is not damaged.

To remove the carousel:

1. Rotate the carousel so that Quadrant 1 is on the front side of the machine.
2. Unscrew the black central knob (Figure 19) by rotating counterclockwise until the threads have disengaged.
3. Lift out the carousel.

Figure 19 – Close-up of knob used to fasten the carousel to the bearing hub



To replace the carousel within the instrument:

1. Rotate carousel so that Quadrant 1 is on the front side of the machine.
2. Place the carousel over the cylindrical bearing hub.
3. Align the holes around the knob with the pins on the bearing hub by slowly rotating the carousel.
4. Screw the central handle clockwise until tight. It is important to tighten the knob fully so that the carousel stays level when the robotic arm pushes down on it to pick up pipette tips.

2.2.10 Pause and Abort Functions

Hitting the “Pause” button will cause RoboSep™ to interrupt the run as soon as it completes the current action. Pressing the “Resume” button will restart the run at the next scheduled action after RoboSep™ orients itself by performing homing actions. The “Abort” button also stops execution of the run as soon as the current action is complete, but with the option to exit from the protocol completely or to resume the run.

2.3 INSTRUMENT CARE

If no more experiments are to be performed that day, or if there was a spill on the instrument, clean the instrument as detailed in Section 5 – Care and Maintenance.

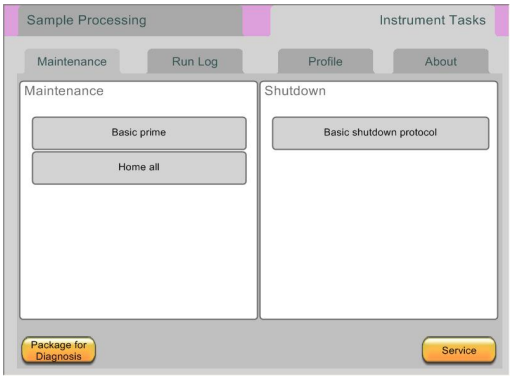
2.3.1 Maintenance Tab

To conserve energy, turn off the instrument at the end of the work day.

- Navigate to the “Instrument Tasks” section of the graphical user interface (Figure 20).
- Select “Basic Shutdown Protocol” from the “Maintenance” tab.
- Wait until the screen becomes black, and then turn the switch on the right side of the instrument to the off position.

Two other protocols can be run from this page: “Basic prime” and “Home all.” If an issue arises, STEMCELL Technologies Technical Support may ask that these protocols be run and the results observed in order to assist with troubleshooting.

Figure 20 – The shutdown protocol, located under Maintenance on the Instrument Tasks tab



Note: The Service software and Package for Diagnosis can be launched from this window, allowing for calibration adjustment and diagnostics. This is not necessary unless requested by a STEMCELL Technologies representative.

2.3.2 Aseptic Operation of RoboSep™

To ensure aseptic operation, RoboSep™ must be used in a biocontainment hood. RoboSep™ is designed to fit easily in most commercially available hoods. In addition, RoboSep™ does not affect sterility within the biocontainment hood; this has been verified by particle count as per ISO standard 14644-1.

Although the hydraulic fluid is internal to the instrument and does not come into contact with the sample, reagents, or tips during normal operation, the use of sterilized deionized water is recommended for aseptic operation.

Standard RoboSep™ protocols dispense fluids into the sample liquid to ensure aerosol containment. Aerosol-resistant filter tips are supplied with each RoboSep™ kit.

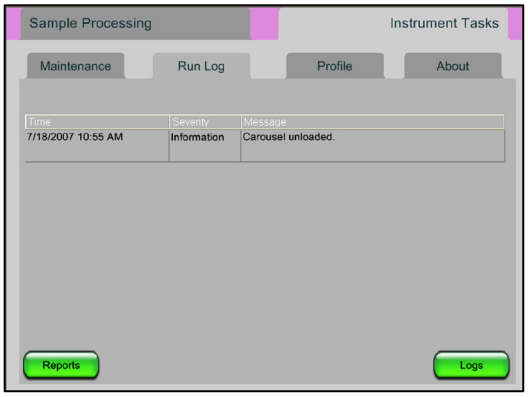
2.4 REPORT FILES

Two types of usage data are automatically saved during RoboSep™ operation: Run Reports and Log Files. Run Reports contain details of each run as entered by the operator, while Log Files contain more detailed information about instrument operation.

2.4.1 End of Run Report Files

Information such as the operator ID, protocol name, run time, reagent lot numbers and usage volume, sample ID and volume is presented in an End of Run Report. To benefit fully from the End of Run Report, the lot number, sample ID and user ID information should be entered during experiment set-up (see Section 2.2.5).

Figure 21 – The Run Log page, shown below, is used to access reports and log files.



2.4.2 Log Files

Log files are used by authorized STEMCELL Technologies personnel to assist with troubleshooting and diagnostics.

2.4.3 Accessing Report Files

The “Run Log” page, under the Instrument Tasks tab, lists diagnostic and run information. The End of Run Report and Log Files are accessed via the buttons at the bottom of the page (Figure 21). Selecting the green “Reports” button brings up a folder containing all of the reports that have been generated to date. These are organized into subfolders by year and month. The Report file name is in the format “Report_DDMMMYYYY_HH_MM_SS.html”. The folder contains all the run reports in chronological order.

Selecting the “Logs” button brings up a folder containing all of the log files created for your instrument. One file is created each time RoboSep™ is initialized. The file names have the format robosep.log.XXX, where “XXX” is “txt” for the most recent file and “XXX” = 1, 2, 3… for increasingly older files.

To access the Report and Log files remotely, set up a connection between RoboSep™ and an external computer as explained in Section 4 – Communications and File Transfer.

3 CUSTOMIZING ROBOSEP™

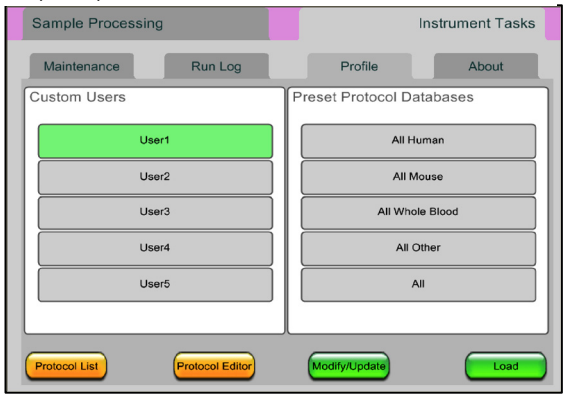
RoboSep™ is provided with a set of protocols optimized for use with “typical” samples. Using the provided RoboSep™ Protocol Editor, users can customize protocols for their specific applications and add them to the global protocol database. Users can also customize the list of protocols that are available to them during an experiment.

Please note: A keyboard and mouse should be connected to RoboSep™ before using the features described in this section.

3.1 PROTOCOL DATABASES

Protocol databases are listed on the Profile page under the Instrument Tasks tab (Figure 22). The Preset Protocol Databases list contains the global protocol list (All) as well as lists based on desired cell species (All Human, All Mouse, All Other) and sample type (e.g. All Whole Blood). The Custom Users list can contain up to 5 user-defined protocol databases.

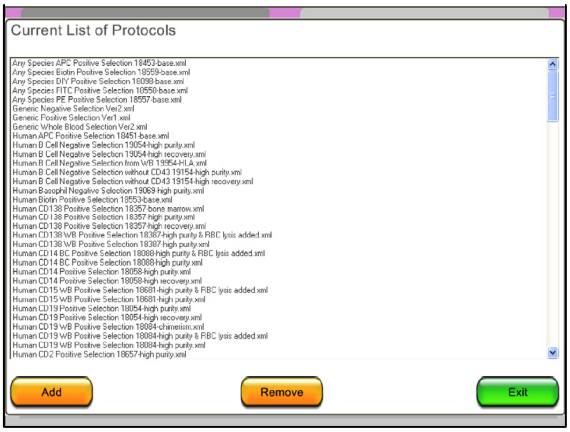
Figure 22 – The profile page displays current custom user profiles and preset protocol databases.



3.1.1 The Global Database

A list of all the available protocols will appear (Figure 23) by selecting the “Protocol List” button. Custom protocols can be added to the list by pressing the “Add” button located at the bottom left of the screen from the Profile page. This will bring up a standard Windows dialog box giving access to protocol files on a USB memory stick or a shared folder on a networked computer. See Section 3.2 for details on creating custom protocols.

Figure 23 – List of all currently available protocols



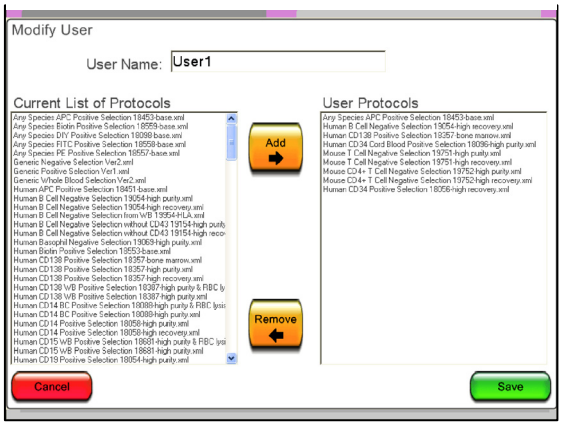
To remove protocols from the global database, highlight a protocol and select the “Remove” button. The selected protocol is moved from the protocol folder to an archived sub-folder. To retrieve an archived protocol, open the protocol with the protocol editor as described in Section 3.2.2 – Editing an Existing Protocol – and save it in the main protocols directory.

3.1.2 Creating and Modifying Custom Databases

RoboSep™ allows you to create up to 5 custom user protocol databases.

1. Select the “Modify/Update” button under the “Profile” tab. A new screen will appear (Figure 22).
2. Enter the desired User Name. This name will appear in the Custom Users list on the Profile page (Figure 22).
3. Select a protocol from the Current List of Protocols on the left and press the “Add” or “Remove” button, as appropriate (Figure 24). Repeat until the User Protocol list on the right contains all desired protocols.
4. Press “Save.” The User Name is now associated with the list of User Protocols

Figure 24 – Customization of protocols available to a specific user



3.1.3 Loading Databases

Go to the Profile page under the “Instrument Tasks” tab.

1. Select a Custom User or Preset Protocol Database (Figure 22).
2. Press “Load.”

The protocols in that database will now be available for use through the “Sample Processing” tab.

3.2 CUSTOM PROTOCOLS – ROBOSEP™ PROTOCOL EDITOR

The RoboSep™ Protocol Editor is a stand-alone program that is installed on RoboSep™. The editor is used to modify existing protocols or to write new ones.

Note: This program is not required for normal RoboSep™ operation.

3.2.1 Start-Up Protocol Editor

To start the protocol editor, press the “Protocol Editor” button under the “Profile” tab (Figure 22). When the Protocol Editor is initially opened, all the fields are grayed-out. To begin, either “New” or “Open” must be selected from the “File” drop-down menu. Select “New” to begin creating a new protocol or “Open” to edit an existing protocol.

3.2.2 Editing an Existing Protocol

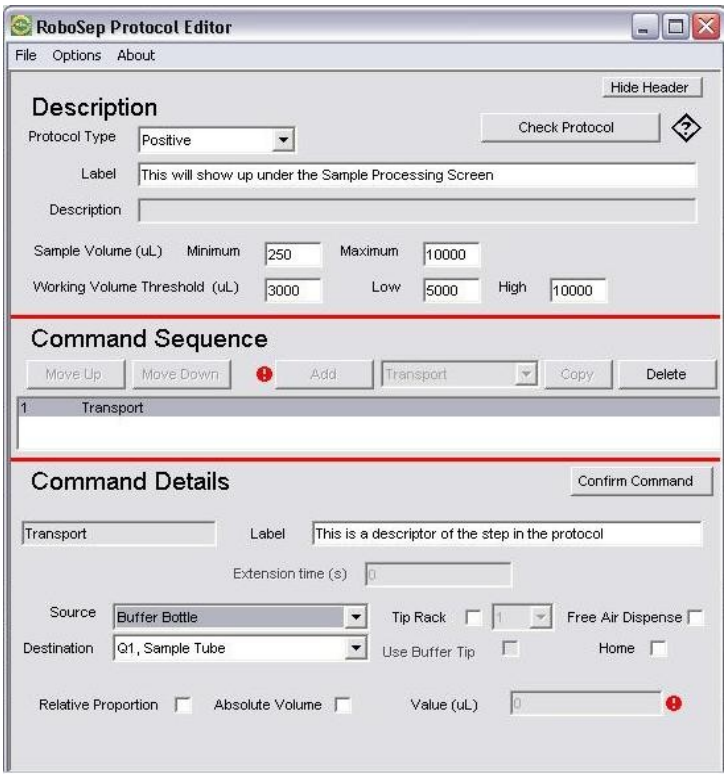
Please contact STEMCELL Technologies Technical Support for assistance if required.

To edit an existing protocol with the Protocol Editor, click on “Open” on the “File” menu and select the desired protocol. This will populate the editor screen with the commands and settings for that protocol. Once the desired protocol is opened, any of its settings and fields can be changed (Figure 25). A description of the commands is found in Section 3.3 – Protocol Structure. When satisfied with the edited protocol, click on “Check Protocol” to ensure that the new values and settings are correct. A screen will pop up if there are errors in the Sample Volume and Working Volume values. Check the volumes highlighted in red on the pop up screen and click “OK” to exit and modify the values on the Protocol Editor. Click on “Check Protocol” again and if the popup screen does not appear, access the “File” menu from the top tool bar and follow the procedures under “Saving a Protocol” (Section 3.4) to save your work.

Notes:

- To avoid over-writing the original protocol, the “Save As” option must be selected instead of “Save.”
- The “About” menu will bring up details of the selected protocol and allow the name of the **Author** to be entered. The Author is for tracking purposes only and does not affect who can use the protocol.
- The **Label** should include a *Protocol Number* in the name. Generally, this is the 5-digit number corresponding to the STEMCELL Technologies catalog number for the product used with the protocol. For more information, see Section 3.3.1.

Figure 25 – Protocol Editor screen fields



1. Protocol Type: Select the desired separation type from the pull-down menu.

2. Label: Enter the name of the protocol as you wish it to appear in the “Protocol Selection” screen on RoboSep™ (see Figure 11); include a protocol number in the name—see Notes above.

3. Description: A short description of the protocol will appear in the Sample Volume dialog box just prior to loading the protocol for a run (see Figure 12).

4. Sample Volume: Enter the minimum and maximum sample volume permitted (see Section 3.3.1 for more information)

5. Working Volume Threshold: Enter the sample threshold volume. RoboSep™ will use the “High” volume for top-up commands with any sample above or equal to the threshold volume. The “Low” volume will be used for top-up commands with any sample smaller than the threshold volumes (see Section 3.3.1).

6. Command Sequence: The list of programmed commands will appear here in the order of execution.

7. Move Up & Move Down: Selected commands can be moved up or down to change the order in which they will be executed.

8. Add: Use the pull-down menu to select the type of command to be added and click the “Add” button.

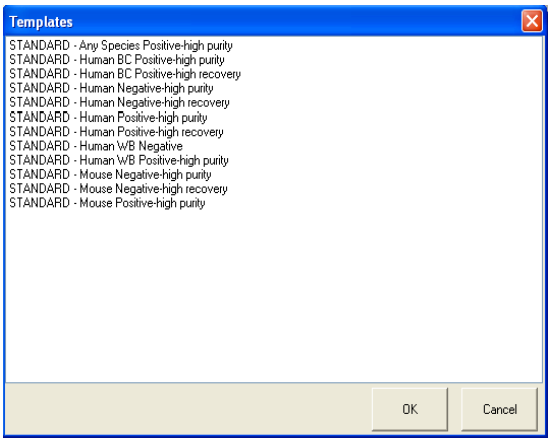
9. Command Details: Parameters that are specific to different commands can be changed here.

3.2.3 Writing a New Protocol

Please contact STEMCELL Technologies Technical Support for assistance if you are using this for the first time.

In the “File” menu, click on “New.” A list of preset standard separation templates will appear (Figure 26). Select the desired separation type from the list and click “OK”. Once the standard separation template is opened, any of its settings can be changed, commands can be added or deleted, and the order of command execution can be changed. A description of the commands is found in Section 3.3 – Protocol Structure. When satisfied with the protocol, access the “File” menu from the top tool bar and follow the procedure under “Saving a Protocol” (Section 3.4) to save your work.

Figure 26 – A list of preset standard separation templates



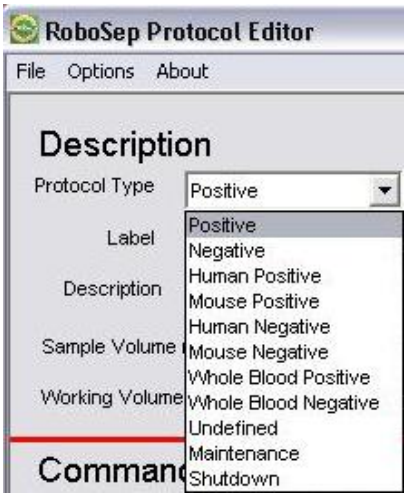
3.3 PROTOCOL STRUCTURE

A protocol is defined by global protocol details, such as positive or negative selection, and by a list of commands with command-specific details, such as incubation time. The global parameters are shown above the first red line in Figure 25, the command sequence is shown below the first red line, and the command-specific parameters are shown below the second red line.

3.3.1 Global Protocol Details

Note: See Figure 25 and Figure 26 for illustration of each point below.

Figure 27 – Protocol type pull-down list



Protocol Type: Select the appropriate protocol type from the pull-down list (Figure 27). The protocol will be listed in the corresponding Preset Protocol Database. Maintenance and Shutdown protocols are for service and diagnostic routines and are not useful for cell separation.

Label: The name entered here is what will appear in the protocol list. This name must be unique for the protocol to be registered as valid. The label should include a *Protocol Number* in the name. This is the 5-digit number corresponding to the STEMCELL Technologies catalog number for the product used with the protocol. Only letters, numbers, and dashes (–) should be used in the label name for proper processing by the RoboSep™ software.

Sample Volume Minimum and Maximum: These fields define the allowable range of sample volumes. Minimum should be set such that all "relative volume" dispensing actions are of at least 12.5 µL. For example: if particles are added at 50 µL/mL of cells, then the minimum sample volume should be 250 µL (12.5 µL x 1000 µL/mL÷ 50 µL/mL= 250 µL).

Maximum should be set such that the total sample volume after all reagent additions is less than 10 mL. For example: if cocktail is added at 100 µL/mL and particles at 50 µL/mL of cells, then the maximum sample volume should be set at 8.5 mL (8.5 mL + (0.1 x 8.5) + (0.05 x 8.5) = 9.8 mL).

Working Volume Threshold (Low and High): After the magnetic labeling steps, RoboSep™ tops up the sample to a given working volume before transferring the sample to the magnet for separation. The top-up volume can be set such that sample volumes less than a given "Working Volume Threshold" are topped-up to a "Low" working volume, while sample volumes greater than or equal to the threshold are topped up to the "High" working volume. This capability exists to improve separation performance for small volume samples.

Suggested settings for negative selection protocols:

- "Working Volume Threshold" = 4000 µL, "Low"= 5000 µL, "High" = 10000 µL.

Suggested settings for positive selection protocols:

- "Working Volume Threshold" = between 1500 and 2000 µL, "Low"= 2500 µL, "High" = 10000 µL.

3.3.2 Protocol Command Sequence and Command-Specific Details

A defined set of commands is used to automate cell separation processes on RoboSep™. These commands are Transport, Mix, Incubate, Top-up/Resuspend, Pause, and Separate. To add a command:

1. Select a command from the pull-down menu in the "Command Sequence" section and click "Add" (Figure 25).
2. Enter the appropriate details (see below).
3. Click on the "Confirm Command" button in the "Command Details" section to confirm the settings.
4. Move command to desired position in command sequence using the "Move Up" and "Move Down" buttons.

The Command Sequence area can be enlarged by using the "Hide Header" button in the top right corner (Figure 25). Each command has specific parameters that influence how it is executed. These will appear in the "Command Details" section of the editor console.

Label: The description in this box will appear in the "Command Sequence" list once all the details of the command have been entered.

Extension Time: Each command takes a set amount of time to execute. The extension time specifies what additional time is available to RoboSep™ to facilitate the scheduling of all the required actions. The default extension times can be changed through the "Options" pull-down menu. See Section 3.3.3 – Advanced Protocol Editor Features for further details.

Source: This is the container (tube, reagent vial, or buffer bottle) from which the pipette will aspirate. If a reagent vial is specified as the source, a 1 mL pipette tip is used automatically. If a sample, separation, or -negative fraction tube is the source, then the 5 mL tip reserved for cell handling is used. If the source is the buffer bottle then the 5 mL tip reserved for buffer handling is used. The source is chosen from a pull-down list which is sorted by relative quadrant number. The source tube should be in Quadrant 1 in most cases, since Quadrant 1 is the start quadrant for all protocols. Tubes in Quadrant 2 are only accessed in protocols requiring two quadrants (e.g. some negative selection protocols).

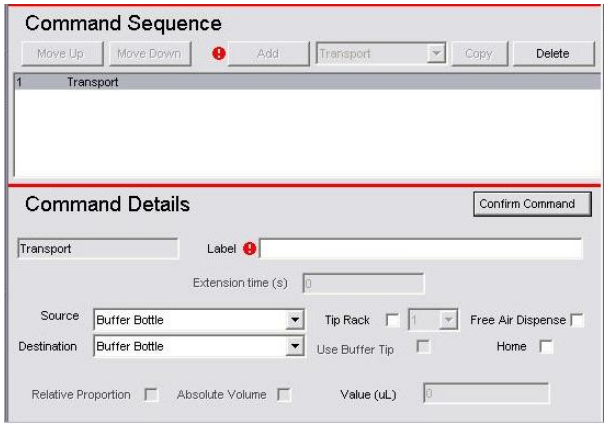
Destination: This is the container into which the pipette will dispense. The destination container is also selected via pull-down menu. The list is sorted by relative quadrant number. A protocol with a source in Quadrant 1 and a destination in Quadrant 2 will occupy two quadrants on the carousel. If this protocol is loaded in Quadrant 3, the destination will be in Quadrant 4.

Free-Air Dispense: When this check box is enabled, RoboSep™ dispenses the fluid that it is transporting just below the upper lip of the destination container and above the liquid in the container. This option is useful where contact with the contents of the destination tube is undesirable (e.g. preventing contamination of the designated buffer tip when adding buffer to the sample tube.).
Note: This option is not recommended for transfer of small volumes (e.g. addition of reagents).

Volume: For Transport commands, the user can specify whether RoboSep™ is to transport an absolute volume (in µL) or a volume relative to the initial sample volume (e.g. to have RoboSep™ add 100 µL of reagent per mL of sample volume, program the addition of a relative volume with a proportion of 0.10).

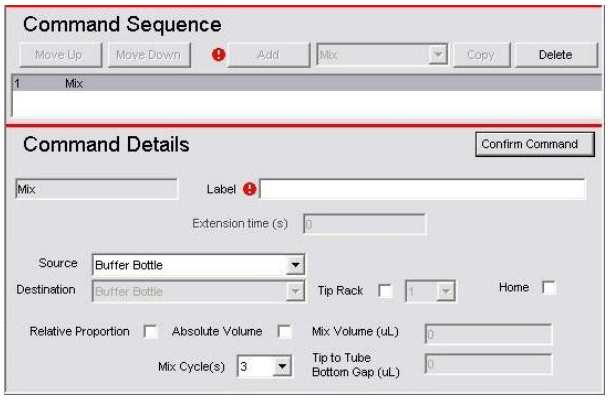
Incubation Time: This parameter specifies the length of time, in seconds, for reagent and magnetic separation incubations.

Figure 28 – Transport command



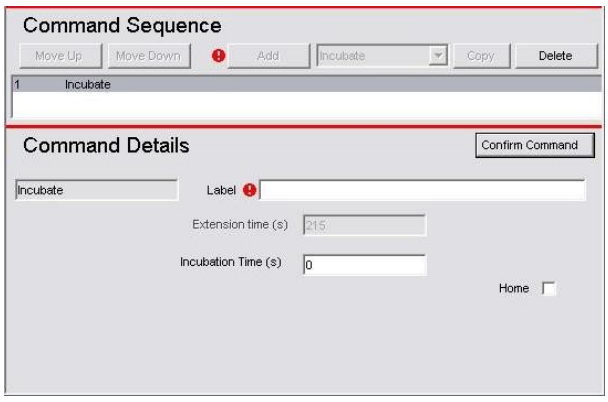
Transport: This command is used to transport fluids from one vial or tube to another (Figure 28). A label is required to describe the steps. The source and destinations need to be appropriately specified, as well as whether a volume relative to the sample volume or an absolute volume is added (see "Volume," above). The default tip rack is in Quadrant 1, but a tip rack in another quadrant can be specified by activating the Tip Rack check box. Free-Air Dispense can also be selected. For negative selection protocols using D magnetic particles or Streptavidin RapidSpheres™, the free-air dispense should be selected when transporting from the sample tube to the selection tube. For all other transports from the sample tube to the selection tube, the free-air dispense should not be selected.

Figure 29 – Mix command



Mix: This command is used to mix the contents of a vial or tube. A label is required to describe the step (Figure 29). The source is the tube containing the sample or reagent to be mixed, and there is an option to specify the extension time (see Section 3.3.3) and the tip rack to be used. There are tip-use rules which decide which tip will be used for each mixing step; these rules are automatically applied by RoboSep™. Selecting "Home" prompts RoboSep™ to perform the "Home All" command (see below) prior to the "Mix" step; this option is not necessary for most RoboSep™ applications. If Relative Proportion is selected, a fraction of the calculated volume in the vial will be mixed. Most standard RoboSep™ mix steps use a relative volume of 0.33. If Absolute Volume is selected, the set volume (entered by the user) will be mixed. The gap between the mixing tip and the bottom of the tube (typically 100 µL) should also be entered. The number of mix cycles can be modified from 1 to 5; most standard RoboSep™ mix steps iterate 3 times.

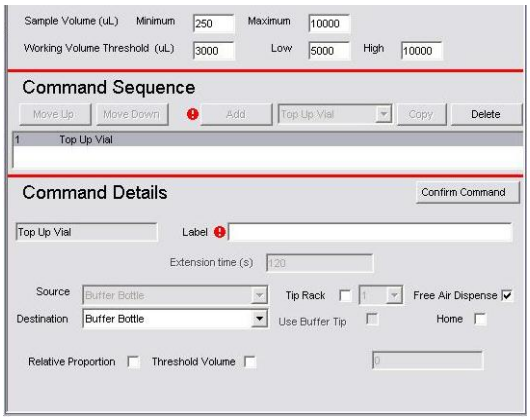
Figure 30 – Incubate or separate command



Incubate: This command allows for incubation of the sample with reagents prior to another step in the sequence being performed (Figure 30). A label is required to describe the step. As before, there are options for extension time and home.

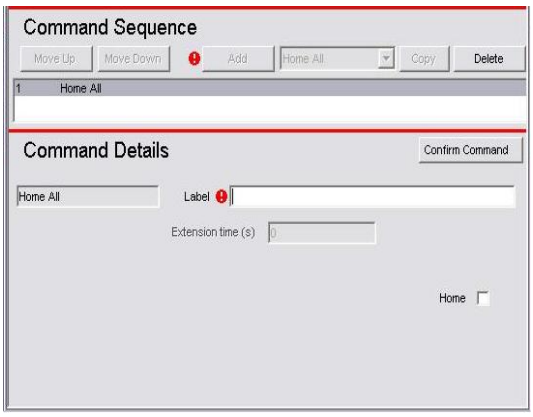
Separate: This command is functionally the same as the "Incubate" command and is used to set incubation times for magnetic separations (Figure 30). The separation time is the minimum amount of time before the next command in the sequence is executed. Typical values for separation time are between 150 and 900 seconds (2.5 and 15 minutes).

Figure 31 – Top-Up command



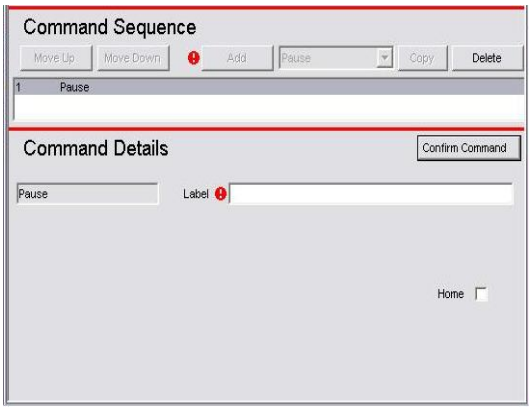
Top-Up and **Resuspend:** "Top-Up" is used to dilute the sample with RoboSep™ buffer before transfer to the magnet for separation (Figure 31). The source is always the buffer bottle. The default top-up volume is specified by the Working Volume Threshold, Low and High values (Figure 25). The top-up volume can also be proportional to the sample volume (e.g. to add 2.0 mL buffer per 1.0 mL sample, enter a relative proportion of 2). This command has the same function as the "Resuspend" command. However, while "Top-Up" is used to bring the sample to the desired working volume at the beginning of the protocol, "Resuspend" is generally used to resuspend cells after magnetic separation steps. Both commands should use a free-air dispense to prevent contamination of the buffer tip with sample.

Figure 32 – Home All command



Home All: This command re-initializes the carousel and robot arm movements (Figure 32). As there is an automatic re-initialization each time RoboSep™ is turned on, or when a protocol is run, this option is not required in standard separation protocols. A label is required and an extension time should be entered to allow the protocol to continue at the end of the "Home All" step (see following section). Alternatively, the components may be left in their homed position (check box).

Figure 33 – Pause command



Pause: The pause command allows the user to intervene at a specific point in a protocol (Figure 33). For example, the pause command could be used if an application requires a centrifugal wash step at some point during processing. The machine will beep and the Label text will appear on the screen when the pause step is reached. Press the "Resume" button when ready to continue.

Flush and **Prime:** These are used internally to prepare the hydraulic fluid system for use. These commands are not needed in cell separation protocols.

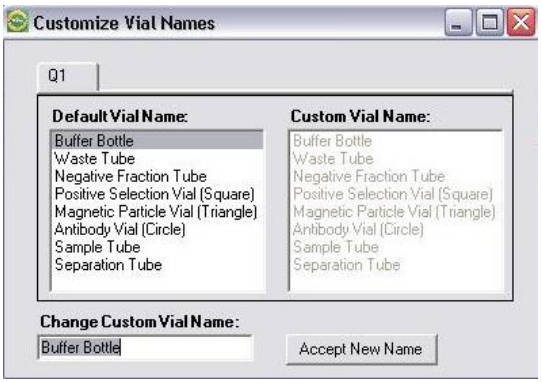
3.3.3 Advanced Protocol Editor Features

Additional capabilities can be accessed through the “Options” pull-down menu (Figure 34).

- By enabling “Extension Time” the user has access to the extension time parameter for all commands. The default extension times have been selected to give maximum flexibility to the built-in protocol scheduler without impacting separation performance. If the default times are altered, for example to allow scheduling of unusual protocols, the desired combination of protocols should be tested in a “wet” run (with water) prior to use in a cell separation experiment to verify that all actions are completed as expected.
- The “Customize Vial Names” menu selection brings up a dialog box that allows the user to change the names of tubes and reagent vials as they appear on the Loading screen. This feature can be used to create names in different languages, or to make the names more appropriate for a given set of custom applications. Highlight the default name you wish to change and enter a new name (Figure 35).
  Note: Names longer than 50 characters may not fit properly on the user interface.
- The “Multiple Samples Selection” menu allows the advanced user to create customized protocols to process multiple samples. Please contact STEMCELL Technologies for more information.

Figure 34 – Options menu

Figure 35 – Customizing vial names



3.4 SAVING A PROTOCOL

The “Save” option will not be available until the protocol is validated by the editor. To do this, click the “Check Protocol” button located at the top-right corner of the editor console (Figure 25). If successful, the symbol beside the “Check Protocol” button will turn from a cross (X) to a tick (√) (Figure 36). The “Check Protocol” function ensures that all information that is critical for protocol execution is present. It does not ensure that your protocol is correctly programmed.

Figure 36 – The Check Protocol dialog (A) before and (B) after a successful protocol check. The file cannot be saved until the check is successful.

3.5 PRINTING A PROTOCOL

Under the “File” menu, there is an option to print out the protocol. This can be used for record keeping, debugging, or troubleshooting with STEMCELL Technologies Technical Support.

4 COMMUNICATIONS AND FILE TRANSFER

Since RoboSep™ runs on Windows XP, it offers many possibilities for communication with other computers. RoboSep™ comes configured for communication directly with another computer via crossover cable (supplied); RoboSep™ can easily be configured to be connected to your network as well.

4.1 SET-UP FOR NETWORK CONNECTION

When a STEMCELL representative installs your instrument, RoboSep™ can be set up to automatically obtain an IP address from the local network. This will allow access to shared folders on RoboSep™ from any other computer on the network. If needed, consult the local network administrator to verify that this is allowed and that the correct permissions are available.

4.2 LOCATING AND CONNECTING TO ROBOSEP™ ON THE NETWORK

Whether RoboSep™ is connected to a network or directly to another computer, it must be located before its shared folders can be accessed by other machines. If using Windows XP, follow these steps to locate the device and view RoboSep™ folders remotely:

1. Select “Search” from the Start Menu.
2. Select “Printers, computers, or people” then select “Computer on network.”
3. Enter RoboSep™ name Robo-XXXX where XXXX is the instrument serial number listed on the side of the instrument or in the “About” tab of the user interface.
4. Double click on the Robo-XXXX icon. You should now see the shared folders on RoboSep™. The shared folders are Protocols, Reports, and Logs.
5. Click on the desired folder and log on as “guest” when prompted.

For instructions on how to mount remote folders in other operating systems, contact the local network administrator.

4.3 ADDING PROTOCOLS REMOTELY

RoboSep™ comes with pre-installed protocols that have been optimized for cell separation from normal samples using supported EasySep™ products. Protocols that have been created for other applications using the protocol editor (see Section 3) can be copied into the Protocols folder on RoboSep™. To do this, navigate to the RoboSep™ folders on the network (as shown in the previous section) and then perform the following:

1. Copy the RoboSep™ protocol(s) from the source computer onto RoboSep™ by either “copying and pasting” or by simply “dragging and dropping” the protocol file into the Protocol Folder.

   Note: The file being copied must have a unique name to avoid potentially overwriting existing protocols. Also, to secure the protocol against accidental modification, change the file properties to “read-only”.
2. Once the desired protocols have been transferred to RoboSep™, update the protocol database list(s) as described in Section 3.1.1.
3. Verify that the protocol behaves as expected with at least one dry run.

Protocols that are no longer required can be removed from the protocol folder and stored in an “Archive” sub-folder on RoboSep™ or on any other networked computer.

4.4 ACCESSING REPORTS AND LOG FILES REMOTELY

For complete information regarding RoboSep™ usage reports and log files, including file structure and naming conventions, please refer to Section 2.4 on page 13. To access these files remotely, locate RoboSep™ on the local network (as described in Section 4.2); the Reports and Logs are found under the appropriately-named folders.

4.5 TRANSFERRING PROTOCOLS VIA USB KEY

1. Insert your USB key into the USB port on your PC. Your PC will detect the USB key as a drive/storage device.
2. Transfer RoboSep™ protocol(s) to or from the USB key by “copying and pasting” or by simply “dragging and dropping”. Remove the USB key from the USB port when the transfer is complete.
3. Insert the USB key into the USB port on RoboSep™. Repeat step 2.

5 CARE AND MAINTENANCE

5.1 GENERAL CONSIDERATIONS

RoboSep™ is designed to avoid any cross-contamination of the samples and reagents manipulated during experiments. In addition, RoboSep™ can be operated within a biocontainment hood to maintain sample and reagent sterility. It is the responsibility of the operator to maintain a clean and uncluttered environment to ensure that RoboSep™ performs as expected.

When using RoboSep™, as during any research experiment, it is essential to observe good laboratory practices as described by your institution or company.

5.2 DAILY MAINTENANCE

The following should be performed daily to ensure longevity and reliability of RoboSep™:

1. Wipe down all exposed surfaces with an alcohol swab.
2. Verify that the hydraulic fluid bottle is full of sterile deionized water.

5.3 CLEANING THE TIP HEAD

At regular intervals, a pop-up screen will prompt you to clean the RoboSep™ tip head. Please use the RoboSep™ Tip Head Polishing Compound (Catalog #20119) provided in the RoboSep™ Accessory Kit to clean the RoboSep™ tip head – *do not use ethanol or isopropanol on the tip head*. The tip head can accumulate a thin film of plastic from repeated loading and unloading of pipette tips; this accumulation will affect the instrument's performance. The RoboSep™ Tip Head Polishing Compound is specially formulated to help remove the build-up of plastic residue and disinfect the tip head.

1. Apply 1 - 2 drops of RoboSep™ Tip Head Polishing Compound to a paper tissue or other laboratory wipe.
2. Wipe the RoboSep™ tip head.
3. Using a new piece of tissue or laboratory wipe, polish the tip head to make sure that all traces of the cleaner are removed.

5.4 CLEANING ROBOSEP™

The preferred method to clean RoboSep™ is to lightly spray 70% ethanol or 70% isopropanol on the instrument's external surfaces, and wipe it off with a soft, clean paper towel. RoboSep™ can also be wiped down with a dilute bleach solution (e.g. 1 in 10 dilution of 5% sodium hypochlorite solution), but should then be wiped clean with water afterwards.

For the touchscreen, it is recommended that the 70% isopropanol or ethanol be sprayed on a soft, clean paper towel prior to wiping down. Excessive direct spray on the touchscreen may cause fluid to seep behind the external screen surface and into electrical components. Do not allow excess fluid to pool at the margins of the touchscreen.

Note: RoboSep™ is resistant to spills; however, it is important to avoid spilling water inside the instrument through openings in the work surface such as around the carousel bearing column and the optical sensor opening.

If accidental spilling of biological material occurs in the carousel area of the instrument and a more thorough cleaning is needed, the following procedure should be used:

1. Unload and remove the carousel (see Figure 19 on page 11).
2. Disinfect the carousel and magnets with a dilute bleach solution and thoroughly rinse clean with water.
3. Rinse with distilled water.
4. Disinfect the spill with dilute bleach solution. Dispose of clean-up material according to local regulations.
5. Rinse the work surface tub with distilled water, then clean with 70% isopropanol or ethanol.

5.5 TIP STRIP FAILURE DETECTION

RoboSep™ has an automatic tip strip failure detector. If the unit detects a tip strip failure, a dialog box will open (Figure 37) and the machine will beep. Pressing the “Resume” button will allow RoboSep™ to try stripping the tip again. Following a successful tip strip, a second dialog box will open (Figure 38). If the tip strip is still not successful, the tip can be removed manually. If a tip strip failure occurs, the tip head may need to be cleaned as described in Section 5.3.

Figure 37 – Dialog box shown if a tip strip failure is detected

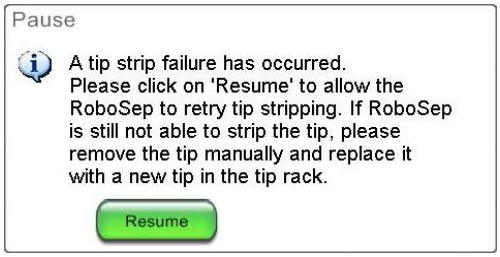
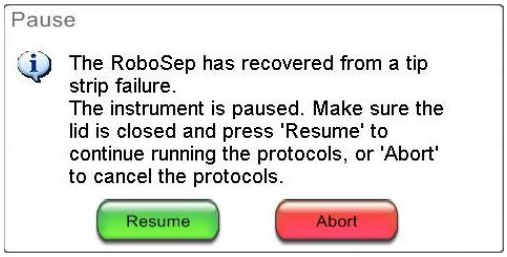


Figure 38 – Dialog box shown following tip strip failure recovery



5.6 REPLACING THE FUSES

The two fuses are located at the bottom right side of the instrument, next to the power supply outlet and the power switch. If RoboSep™ does not turn on when plugged in and the power switch is activated, the fuses may need to be replaced. To replace the fuses:

1. Disconnect the power cable from the power source.
2. Disconnect the power cable from the instrument.
3. Open the fuse box and examine the fuses.
4. Replace the faulty fuse(s). Always use Type F 250V 4A 5X20mm fuses.
5. Reinsert the fuse box containing the new fuse(s) in its slot. Press gently until it snaps into place.
6. Reconnect the power cable to the instrument, then to a grounded (three-prong) power source.

6 TROUBLESHOOTING

RoboSep™ presents error messages to help diagnose some types of problems that may occur during use. Please record the message before calling Technical Support. The following tables address other potential issues that are not flagged as errors.

PROBLEM	POTENTIAL CAUSE	SOLUTION
RoboSep™ screen does not turn on. No sound, movement, or sign of activity within 30 seconds of attempting to power up the instrument.	Faulty fuse.	Check that the fuses are correctly installed in described in Section 5.6.
	No power to outlet.	Check that the outlet is active by connecting a known working device such as a lamp.
Operating system boots up but RoboSep™ fails to fully initialize.	Power switch not left on long enough. It takes up to 10 seconds for the electronics to fully reset after the power switch has been turned off.	After shutdown, turn off the power switch and count to 10 before turning the power back on again.
RoboSep™ won't resume a paused run.	Lid up. RoboSep™ will not run if the lid is in the raised position.	Lower the lid and press Resume on screen.
RoboSep™ won't shut down because the basic shutdown protocol won't load.	Previous run not complete or needs to be unloaded.	Let run finish. Press unload button on the Sample Processing screen.
Reagents left in tips.	RoboSep™ leaves a residual volume in tips when pipetting small volumes.	This is a normal operation and is required to meet the accuracy requirements for low volume reagent addition. The residual volume is 20 µL or less.
Missed tip pick-up.	Tip rack not placed properly on the carousel.	Make sure that the tip rack is seated properly by pushing down firmly on the outside of the exposed portion.
	Robotic arm or carousel was bumped during operation.	Only operate with the lid down. *If the above measures fail to solve the issue, contact Technical Support.*
Tip head spits hydraulic fluid.	Hydraulic fluid line contamination.	Fill hydraulic fluid bottle with 70% isopropanol or 70% ethanol and prime >4 times. Leave alcohol in line for >1 hour (longer times preferred), prime >4 times with sterile deionized water. If problem persists, contact Technical Support.
	Damage to fluid line.	Contact Technical Support.
Tips not well aligned with tubes.	Positional calibration is required.	Contact Technical Support.
Tip rack separates at the snap joint when removing cap.	Excess force applied at this joint by mistake.	Ensure only the top cap is removed.
Missed tip stripping action.	Tip stripping arm not moving.	Arm is out of position. Power down the instrument, open the lid, and pull the tip stripping arm out. Restart RoboSep™.
	Tip stripping arm misaligned.	Contact Technical Support.
Priming step missed or incomplete: No water ejected from the tip head prior to run.	Connections in hydraulic system are leaking air.	Make sure the connection between the hydraulic fluid bottle and tubing is seated correctly by disconnecting and reconnecting firmly.
	Hydraulic fluid bottle is empty.	Refill bottle.
Can't load protocol in Protocol Editor.	File is read-only.	Change file property.
Custom protocol does not appear in protocol database.	File label is not unique.	Create unique label for protocol using Protocol Editor (see Section 3.3).

7 SPECIFICATIONS

Capacity	- Labels and separates up to 4 samples simultaneously
	- Up to 8 x 10^9 total cells (four samples of up to 2 x 10^9 cells each)
	- Sample volume: from 250 µL to 8.5 mL for each sample
	- Pipetting range: 12.5 µL to 5 mL (accuracy: volume variations <10% over whole pipetting range)
Dimensions and weight	- Height with lid: 56 cm (22 in)
	- Width: 70.7 cm (27 in)
	- Depth: 39.2 cm (15 in)
	- Weight: 32.6 kg (72 lb)
Power Requirements	- Electrical supply 100-120/220-240 V AC, 50/60 Hz, 260 W
	- Uses two Type F 250V 4A 5X20mm
Conditions for Operation	- Temperature 10°C to 30°C
	- Relative humidity 20 - 85% (non-condensing)
	- RoboSep™ is not specified to be used in a cold room (4°C, 39°F)
	- For indoor use only
Certifications	- Pollution degree 2
	- Installation category II
	- Altitude 2000 m
	- CSA certificate 1660865
	- UL 61010-1
	- CE marked

- RoboSep™ is designed and manufactured so that it does not endanger the safety of human operators when properly installed and maintained and used in applications for which it was intended. RoboSep™ meets the requirements of EC directive 2006/95/EC (low-voltage directive) and 2004/108/EC. RoboSep™ meets the requirements of CAN/CSA-C22.2 No. 61010.1-04 and UL standard No. 61010-1 (safety requirements for electrical equipment for measurement, control and laboratory use).
- The RoboSep™ unit has a symbol affixed to the side, similar to the following: 

  This symbol denotes that the device must be disposed of in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive.
- RoboSep™ has been designed for easy dismantling and is in compliance with the recovery, reuse, and recycling targets set out in the WEEE Directive.
- RoboSep™ should not be disposed of at civic disposal sites.
- Please contact STEMCELL Technologies for information about the meaning of the WEEE symbol and for information about return and collection points for the device at the end of its life.

7.1 FACTORY CALIBRATION

Dispensing, robotic positioning, and the touchscreen used on RoboSep™ are all factory calibrated. Adjustments to any settings should be performed only by qualified personnel.

7.2 SECURITY

Secured access to RoboSep™ can be implemented by connecting RoboSep™ to your institution's network. These approaches prevent unauthorized access to the RoboSep™ software, and may require IT support. Additional user profiles can also be set up on stand-alone units, as RoboSep™ operates on a Windows XP platform. This is not the standard set-up; contact STEMCELL Technologies Technical Support for additional information. A fee for secured instrument set-up may apply.

8 ROBOSEP™ SPARE PARTS AND ACCESSORIES

PRODUCT NAME	CATALOG #
RoboSep™ Service Rack	20101
Hydraulic Fluid Bottle and Tubing	20102
RoboSep™ Buffer (250 mL)	20104
5X RoboSep™ Buffer (250 mL)	20124
EasySep™ RBC Lysis Buffer 10X Concentrate (100 mL)	20120
RoboSep™ Filter Tip Racks (1 box of 8 racks)	20125
RoboSep™ Tip Head Polishing Compound (7 mL)	20119
Ethernet Cross-over Cable	20106
RoboSep™ Carousel	20107
“The Big Easy” EasySep™ Magnet	18001
RoboSep™ Barcode Scanner	20501

9 WARRANTY

The following is an overview of the warranty and service options available for RoboSep™. For answers to any maintenance or warranty questions, please contact STEMCELL Technical Support (contact information is available in Section 10).

WARRANTY PLAN	COVERAGE	COVERAGE DETAILS	CATALOG #
RoboSep™ Limited Warranty[1]	1 Year[2]	• Parts • Labor	20200
RoboSep™ Limited Warranty with Preventative Maintenance Package	1 Year[2]	• Parts • Labor • Preventive Maintenance[3]	20202
Preventative Maintenance Package	1 Year[2]	• Preventive Maintenance	20203

[1] Included in purchase of RoboSep™.

[2] Maximum purchase of up to 5 consecutive years.

[3] Preventative Maintenance includes: Full calibration check, touchscreen verification, tip head verification, tip stripping verification, hard-ware verification, soft-ware verification and/or upgrades, robot homing verification, fluidic system verification, electrical card and connector verification, pump and drive verification, fastener and tubing verification, belt and motor verification, bearing and gear verification, verification certificate, related shipping costs (if applicable).

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