Celselect Slide™ Enrichment User Manual

Introduction

Celselect Slides™ capture and isolate individual circulating tumor cells (CTCs) based on their size, utilizing microfluidics and 56,400 individual microchambers. Isolated cells can be retrieved for downstream analysis such as single-cell genomics, digital PCR (dPCR) or Flourescence In Situ Hybridization (FISH).

Fig. 1. The Genesis System.

Precautions

- Do not use reagents and consumables beyond the expiration date on the label
- Visually inspect each blood sample for clots before processing on the Genesis System. Clotted samples should be discarded
- Protect reagents from exposure to light sources
- Do not mix and match reagents from different lots/batches
- This user manual is designed for use with the Genesis System
- Protective barriers such as gloves, gowns, masks, and eye protection should be utilized when working with whole blood specimens

List of Provided Components and Consumables

The following components and consumables are required to complete the Celselect Slide Enrichment Assay and are available from Bio-Rad™ Laboratories, Inc.:

Table 1. Celselect Slide Enrichment Kit (catalog #CEL80110). Each kit supports processing of 4 samples.

Box 1 of 2: Shipment and Storage Conditions: Room Temperature	
Celselect Consumables	**Quantity**
Celselect Slide	4
Reagents Cartridge Piercer	4
Celselect Slide Manifold	4
Waste Jars & Tubing	4
Inlet Funnel	4
Priming Reservoir	4
Box 2 of 2: Shipment and Storage Conditions: 4°C	
Celselect Reagents	**Quantity**
Dilution Buffer	4
Reagents Cartridge	4

Celselect Slide and Consumables

Note: Each of the Celselect Slides and Consumables are intended for single-use.

The Celselect Slide (Figure 2) is Bio-Rad proprietary CTC capture and analysis technology. The slide has one laminated side and the other side contains inlet and outlet ports. Optical characteristics closely mimic a glass slide, rendering the slide fit for imaging.

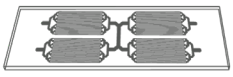


Fig. 2. Celselect Slide.

The disposable Celselect Slide Manifold (Figure 3) holds the Celselect Slide. Each Manifold has a slide slot and an inlet and outlet port. The U-shaped cutout at one end of the manifold and arrows designate the outlet direction.

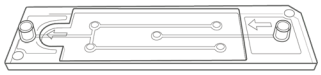


Fig. 3. Celselect Slide Manifold.

Blood and other reagents enter the manifold through the Inlet Funnel (Figure 4).

Fig. 4. Inlet Funnel.

The Reagents Cartridge Piercer (Figure 5) is firmly placed in the drip plate of the Genesis System to penetrate the bottom of the Reagents Cartridge and allow reagents to enter the Inlet Funnel.

Fig. 5. Reagents Cartridge Piercer.

The Waste Jar and Tubing (Figure 6) collect reagents after processing.

Fig. 6. Waste Jar and Tubing.

The Genesis Priming Reservoir (Figure 7) is used during priming and again once the cells have already been isolated.

Fig. 7. Priming Reservoir.

Celselect Slide Reagents

Note: These reagent solutions are dispensed in single aliquots with an entire set provided for each run.

Dilution Buffer

The Dilution Buffer is used to retrieve the cells after washes while promoting proper flow properties and viability with the cells during the protocol.

Reagents Cartridge

The Reagents Cartridge is shipped pre-loaded with all necessary reagents to facilitate cell capture and purification.

List of Equipment Provided with the Genesis System

Table 2. Equipment provided.

Equipment	Catalog Number	Quantity
Celselect Slide Station	CEL84085	2
Reagents Cartridge Venter	CEL84093	1
Celselect Slide Imaging Adaptor	CEL84083	1
Wireless Barcode Scanner	CEL84092	1
Backflow Adaptor	12016521	2

The Celselect Slide Station (Figure 8) is composed of a base and a clamp and is designed specifically to orient and stabilize the Celselect Manifold and Slide on the Genesis System.

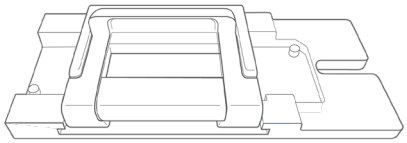


Fig. 8. Celselect Slide Station.

The Celselect Slide Imaging Adaptor (Figure 9) can hold up to four Celselect Slides and is designed to fit directly in the Agilent Lionheart Automated Microscope. It replaces and can be used interchangeably during Celselect Slide Enumeration with the adaptors provided by Agilent with the Lionheart LX System (recommended).

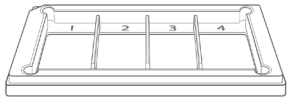


Fig. 9. Celselect Slide Imaging Adaptor.

The Reagents Cartridge Venter (Figure 10) is designed to fit directly onto, and open, the chambers of the Reagents Cartridge for its use on the Genesis System. The Cartridges require this piercing in order to prevent the formation of a vacuum and allow proper flow of the contained reagents.

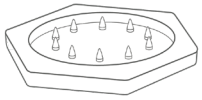


Fig. 10. Reagents Cartridge Venter.

The Backflow Adaptor (Figure 11) is designed to connect the Genesis System vacuum pump to the Priming Reservoir, producing positive pressure to prime Slides and retrieve captured cells during CTC Enrichment Assays.

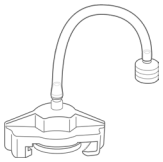


Fig. 11. Backflow Adaptor.

User-Supplied Equipment, Consumables, and Reagents

The following are additional equipment, consumables and reagents necessary to perform Celselect Enrichment.

Table 3. Equipment required but not provided by Bio-Rad.

Supplier	Description
Any vendor	Single-channel pipettes (20 µL, 200 µL, and 1000 µL)
Any vendor	Serological pipette controller
Any vendor	Test tube rocker
Any vendor	Test tube rack

Table 4. Consumables and reagents required but not provided by Bio-Rad.

Supplier	Description
Any vendor	5 mL and 15 mL conical tubes
Any vendor	Sterile disposable serological pipettes (5 mL, 10 mL)
Any vendor	Phosphate buffered saline (PBS) 1X, pH 7.4
Any vendor	Pipette tips (20 µL, 200 µL, and 1000 µL)
Any vendor	70% ethanol solution

Performing the Genesis System Self Test

1. Power on the Genesis System using the switch found on the back bottom left corner of the instrument (Figure 12).

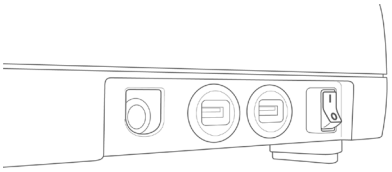


Fig. 12. Genesis System back panel.

2. Once the instrument is on, the Genesis GUI (Graphical User Interface) home screen loads automatically after boot and logs every run. From here, one can log in, gather system information or shut down the instrument.

Note: Log in is not required.

Fig. 13. Genesis GUI home screen.

3. Place Waste Jar in position at the base of the station bays nearest the user (Figure 14).

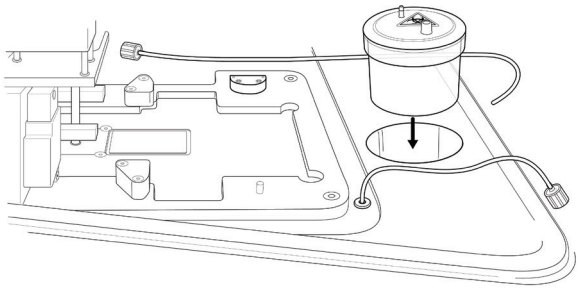


Fig. 14. Place waste jar in position.

4. Attach the waste jar tubing to the Waste Jar (Figure 15).

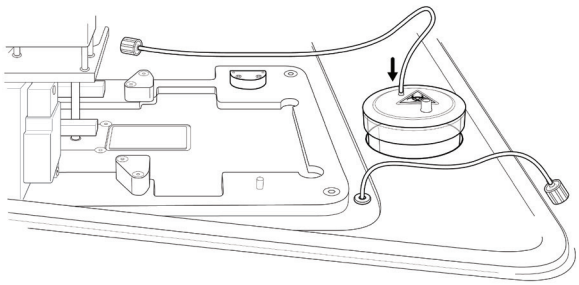


Fig. 15. Attach waste jar tubing.

5. Thread tubing through the pinch valve (Figure 16).

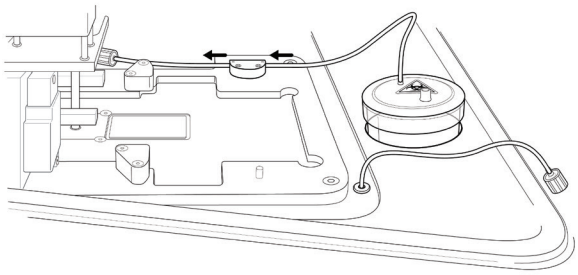


Fig. 16. Thread tubing through pinch valve.

6. Attach vacuum tube to the Waste Jar outlet (Figure 17).

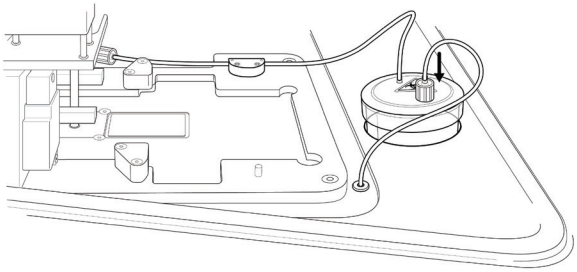


Fig. 17. Attach vacuum tube.

7. Press “Start” from the GUI home screen to reach Self Test screen. Select each lane to initiate the “Self Test” (Figure 18).

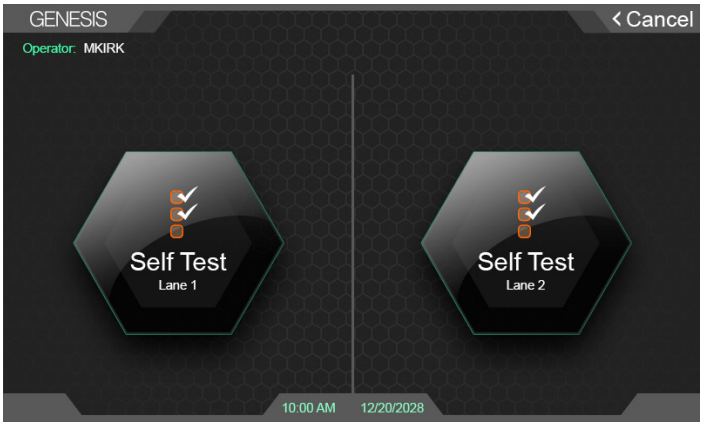


Fig. 18. Run Self Test.

8. A confirmation list will appear on the screen, which lists the following actions/items the system looks for PRIOR to running the Self Test (Figure 19).

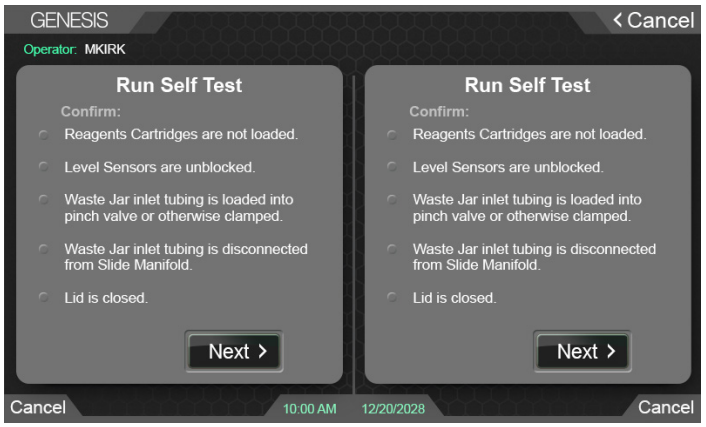


Fig. 19. Confirm Run Self Test.

9. Ensure the configuration is correct, then click “Next” to initiate Self Test protocol.

10. When the Self Test is complete, select the Enrichment option from the protocol dropdown list (Figure 20).

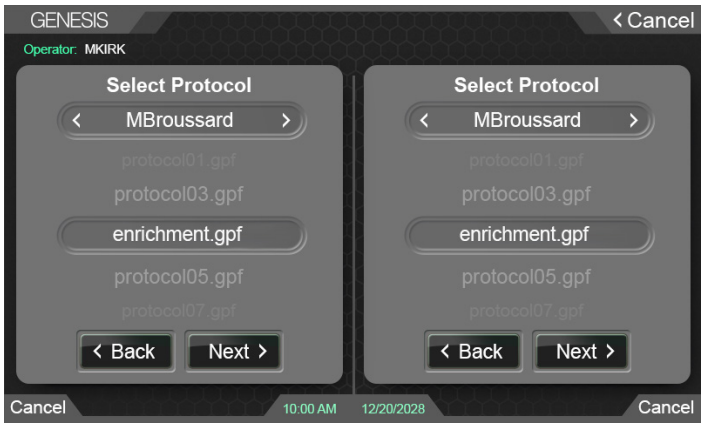


Fig. 20. After a successful Self Test, the protocols can be selected.

11. Populate the data entry fields recording the reagents, consumables and slide identifiers using the Barcode Scanner (Figure 21).

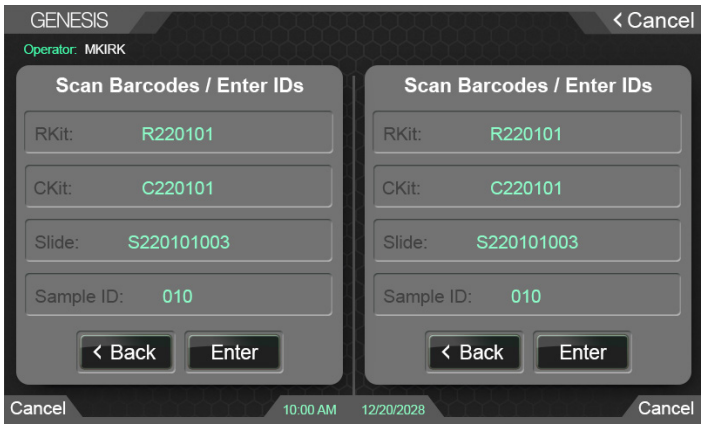


Fig. 21. Populate data for run.

Note: If you are unable to scan barcode/QR code, use the following to manually enter the lot number identifiers:

a. Celselect Slides Enrichment Reagents (4 Pack): “R” followed by the 6 digit lot number, e.g., R010101

b. Celselect Slides Enrichment Consumables (4 Pack): “M” followed by the 6 digit lot number, e.g., M010101

c. Celselect Slides (4 Pack): “S” followed by the 9 digit serial number, e.g., S010101001

d. The “Sample ID” field is a freeform field allowing the user to define a unique sample name for the run.

Celselect Slide and Slide Station Set-Up

Note: If 70% ethanol was acquired or pre-made no additional steps are required. If it is being prepared from concentrate for this experiment, do so at this and allow it to rest at room temperature for at least 1 hour prior to beginning the assay.

1. Place the Slide Manifold onto the Slide Station using the two positioning pins. The arrows on the manifold should point to the left when the Slide Station is oriented horizontally with the clamp on the opposite side to the user (Figure 22).
2. Place the Celselect Slide label-side up on top of the manifold, aligning the ports of the Celselect Slide within the five overmolded O-rings on the manifold (Figure 23).
3. Engage the clamp to seal the Celselect Slide between the manifold and the top cover (Figure 24).
4. Insert the Inlet Funnel into the inlet port of the manifold. Make sure the Inlet Funnel is completely inserted before proceeding to the priming steps (Figure 25).

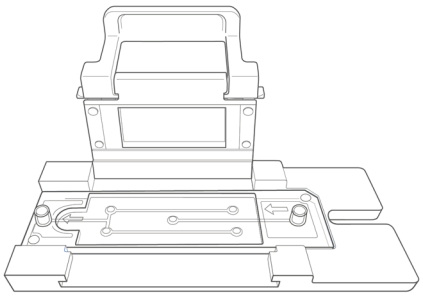


Fig. 22. Manifold loaded into the Celselect Slide Station.

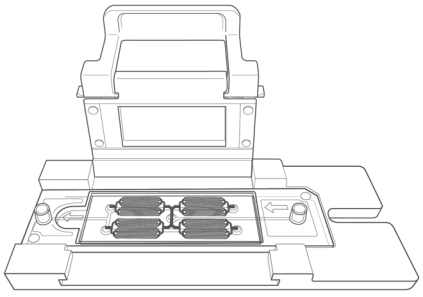


Fig. 23. Celselect Slide label-side up.

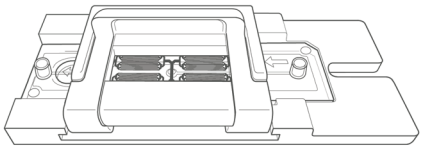


Fig. 24. Top Cover clamped on Celselect Slide and Slide Manifold.

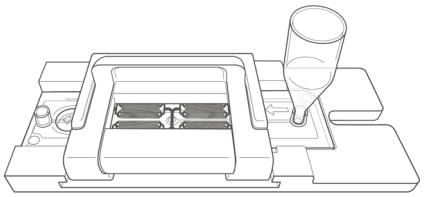


Fig. 25. Inlet Funnel in Slide Manifold inlet port.

Priming the Celselect Slide

Note: Please mix the 70% ethanol solution and allow it to rest at room temperature for at least 1 hour prior to beginning the assay.

1. Add the assembled slide station(s) to the Genesis (Figure 26).

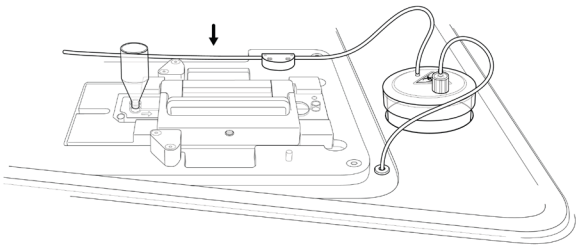


Fig. 26. Add the assembled slide station.

2. Insert the Genesis Priming Reservoir into the outlet port of the assembled slide station (Figure 27).

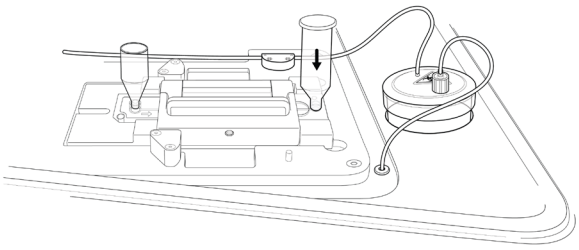


Fig. 27. Insert the Genesis Priming Reservoir.

3. Attach the Backflow Adaptor to the Genesis Priming Reservoir (Figure 28).

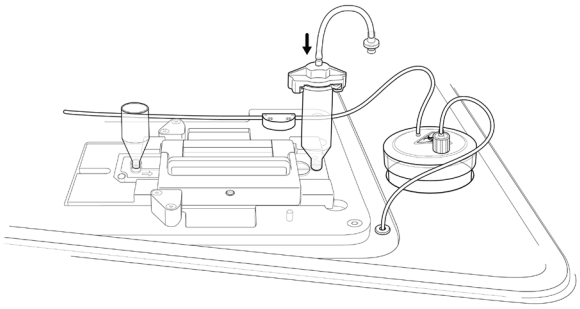


Fig. 28. Attach the Backflow Adaptor.

4. Attach the vacuum tubing from the Genesis to the Backflow Adaptor tubing (Figure 29).

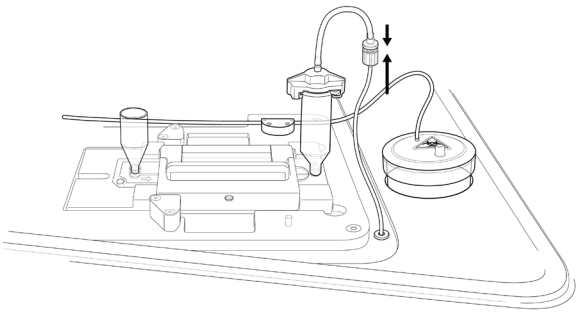


Fig. 29. Attach the vacuum tubing to the Backflow Adaptor tubing.

5. Add 8 mL of room temperature 70% ethanol solution to the inlet funnel using a 10 mL pipette.

6. The instrument GUI will prompt the user to confirm the proper data for the run was entered (Figure 30).

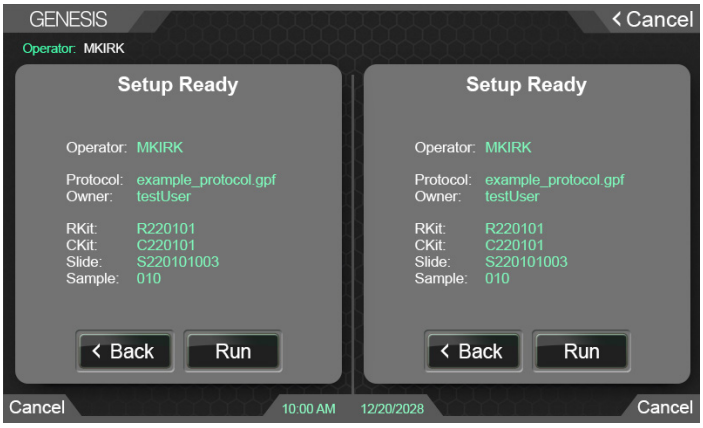


Fig. 30. Confirm run data.

7. Ensure the run data is correct, then select “Run” to initiate the protocol. If any item is not correct, resolve the item(s). Select “Back” to re-enter the information.

8. Hit the “Run” button, and the priming protocol will begin.

9. The first part of the priming protocol will take approximately 15–20 minutes.

10. When the first half of the priming is complete, a prompt will appear on the screen stating, “Attach waste jar and add 8 mL of 1X PBS to inlet funnel.”

Note: Do not proceed or click “OK” until the instructed actions in steps 12–18 are complete.

11. When prompted, disconnect the Backflow Adaptor from the Genesis vacuum tubing.

12. Remove the Backflow Adaptor from the Priming Reservoir.

13. Remove all of the ethanol from the Genesis Priming Reservoir using a 10 mL pipette.

14. Remove the Genesis Priming Reservoir from the manifold. Gently blot the Priming Reservoir with a wipe (or lint free cloth) and tap the Priming Reservoir to the wipe on a hard surface to remove excess ethanol. Let the Priming Reservoir stand to air dry.

15. Attach the waste jar tubing to the outlet port of the manifold in the assembled slide station.

16. Connect the Genesis vacuum tubing to the top of the waste jar (Figure 31).

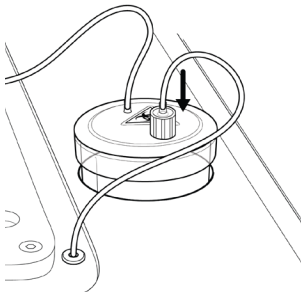


Fig. 31. Connect the vacuum tubing to the top of the waste jar.

17. Add 8 mL of 1X PBS to the inlet funnel.

18. Press “OK” on the prompt.

19. The final step of priming will commence. This step will take approximately 2–5 minutes.

Preparing the Reagents Cartridge

Note: The Reagent Cartridges and Dilution Buffer must to be warmed at 37°C for 10 minutes prior to use and left at room temperature.

1. Pierce the top foil of the Reagents Cartridge with the Reagents Cartridge Venter, making sure to twist the Venter to open the foil (Figure 32).

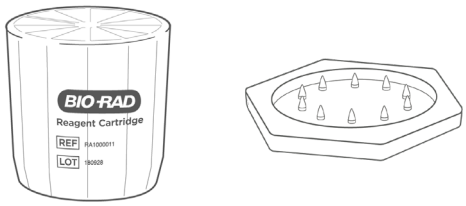


Fig. 32. Reagents Cartridge and Reagents Cartridge Venter.

Preparing the Blood Sample

Note: Reference Appendix A for Specimen Collection, Transport, and Storage.

1. Label 15 mL conical tubes for each sample.
2. Pipette 4 mL of blood from the specimen tube into its respective 15 mL conical tube.

Note: If performing a spike-in experiment, add your target cell population here. See Appendix B: Cell Line Spike-In Guidelines.

3. Add 4 mL of 1X PBS to the sample and gently mix by inverting the tube 3–5 times.

Note: Shaking too vigorously can introduce air into the sample. This air can escape during the run and cause clogs in the Slide.

Running the Genesis System

1. Add 8 mL of sample to Inlet Funnel of each Celselect Slide Station (Figure 33).

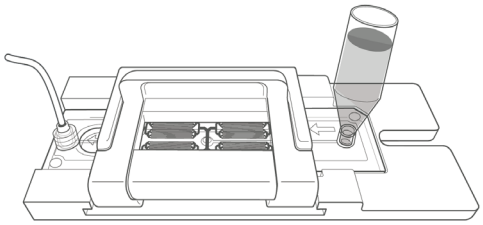
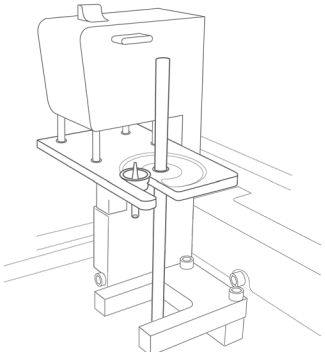


Fig. 33. 10 mL of prepared blood sample added to Inlet Funnel on Celselect Slide Station.

2. Load the Reagents Cartridge Piercer into the drip plate until it snaps into place (Figure 34).



3. Fig. 34. Reagents Cartridge Piercer snapped in place on drip plate.

4. Pull aside the cartridge retainer arm and place the Reagents Cartridge on the spindle, spinning it slowly until the keys align and it drops down (Figure 35).

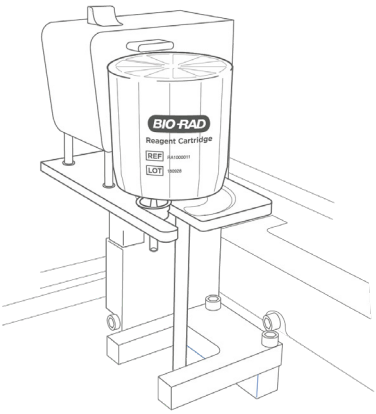


Fig. 35. Reagents Cartridge on spindle.

5. Release the cartridge retainer arm to its original position over the Reagents Cartridge.
6. Select “OK” on the User Action to initiate the rest of the run
7. Once the system has completed filtering the sample, the system will prompt the user to proceed to backflow to retrieve the isolated cells.

Note: Do not proceed to the backflow until steps 9–13 are complete.

8. Remove the Waste Jar Tubing from the manifold.
9. Place the Priming Reservoir in the outlet port of the manifold.
10. Add 4 mL of Dilution Buffer to the Priming Reservoir.

11. Attach the Backflow Adaptor to the Priming Reservoir (Figure 36).

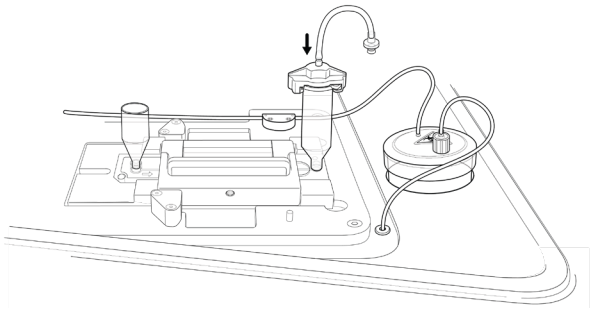


Fig. 36. Attach the Backflow Adaptor.

12. Attach the vacuum tubing to the Backflow Adaptor (Figure 37).

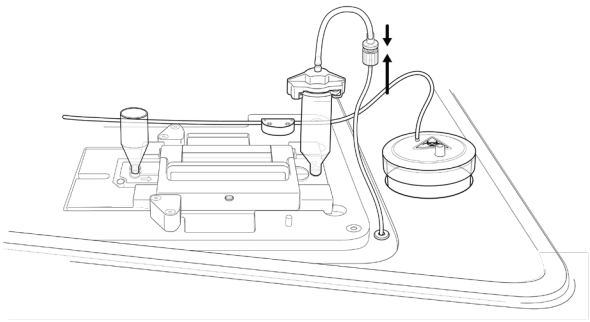


Fig. 37. Attach the vacuum tubing to the Backflow Adaptor tubing.

13. Select Start Backflow and allow the Genesis Pump to run until all of the buffer has passed through the Celselect Slide into the Inlet Funnel.
14. Press Finish.
15. Collect the enriched sample from the inlet funnel in a labeled 15 mL conical tube and store at 4°C. The sample can be centrifuged at 400 RCF for 7 minutes to concentrate the cells.
16. Remove the vacuum tubing from the Backflow Adaptor.
17. Remove the Backflow Adaptor from the Priming Reservoir.
18. Dispose of the Waste Jar and Tubing, the Priming Reservior, the Reagents Cartridge, the Cartridge Piercer and the Inlet Funnel.
19. Remove the Slide Station.
20. Disassemble the Slide Station by unclamping the cover and removing the slide and manifold, and disposing of the consumables.

Cleaning the Genesis System

Note: Clean the Genesis System after each use.

1. Wipe instrument deck with 10% bleach solution on a towelette.
2. Wipe instrument deck with 70% ethanol on a towelette.

Cleaning the Slide Stations

Note: Clean the Slide Stations after each use.

1. Wipe Stations with 10% bleach solution on a towelette.
2. Wipe Stations with 70% ethanol on a towelette.

Appendix

A. Specimen Collection, Transport, and Storage

Collect blood aseptically into EDTA tubes.

Caution: Once the tube is opened, process blood immediately.

Table 5. Specimen collection, transport, and storage.

Enumeration Protocol	Transportation Temperature	Storage Temperature	Shelf Life
EDTA tubes	Ship on ice to increase the stability of the cells.	Refrigerate sample and bring to room temperature 15–30°C (59–86°F) before processing.	Process within 24 hours after collection.

B. Cell Line Spike-In Guidelines

Note: Below is an approach to conducting a “spike-in” experiment using a CTC cell line and blood for training on this protocol.

1. Wash flask of MCF7 cells with 1X PBS (Example: 3 mL for T25 flask).
2. Add 0.25% Trypsin to flask and incubate at 37°C for 5 minutes (Example: 3 mL for a T25 flask).
3. Add an equal volume of media to “neutralize” Trypsin.
4. Transfer cell suspension solution and add to a 15 mL conical tube.
5. Transfer 10 µL of cell suspension and add to 1.5 mL centrifuge tube.
6. Add 10 µL of Trypan Blue to centrifuge tube and mix with cell suspension.
7. Count cells with 10 µL of the Trypan Blue/cell mixture on a hemocytometer.

8. Centrifuge cell suspension in 15 mL conical tube at 200xg for 5 minutes.
9. Resuspend cells at 10,000 cells/mL of PBS (this may require two or more serial dilutions).
10. Add 50 µL of suspended cells to 4 mL of a whole blood sample.
11. Add 4 mL of PBS and and gently mix by inverting the tube several times.
12. Return to “Running the Genesis System,” step 1.

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Life Science Group

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