Celselect Slide™ CTC Enumeration Direct Staining User Manual

Introduction

Celselect Slides™ are used with the Genesis System to capture and isolate individual circulating tumor cells (CTCs) based on their size, utilizing microfluidics and 56,400 individual microchambers. Isolated cells can be stained for immunofluorescent applications such as enumeration and Flourescence In Situ Hybridization (FISH), or enriched for downstream analysis such as cell culture or single-cell genomics. After staining, the cells are counted on an automatic imager such as the Agilent Lionheart LX Automated Microscope (recommended). The high capture efficiency and customizability paired with excellent sensitivity and specificity make Celselect Slides the ideal approach to follow tumor progression and response to therapy. The Genesis System and Celselect Slide Technology are used together to support users who want to optimize, modify, or create new protocols or custom reagent formulations.

There are two approaches used to stain and identify CTCs. One utilizes indirect staining where primary antibodies attach to cell-specific surface antigens and are subsequently bound by secondary antibodies that fluoresce at amplified levels. The second approach, direct staining, utilizes a conjugated antibody that binds and fluoresces specifically at the antigen. Essentially, direct staining achieves higher specificity at the cost of lower fluorescent signal intensity. Both methods permeabilize the cell membrane, facilitating the use of additional cytosolic markers, as well as surface markers. This user manual is for direct staining. For indirect staining, please see Celselect Slide CTC Enumeration Indirect Staining User Manual (3338).

Fig. 1. The Genesis System.

Precautions

- Do not use consumables and reagents 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

BIO-RAD

List of Provided Components and Consumables

The following components and consumables are required to complete the Celselect Slide CTC Enumeration Direct Staining Assay and are available from Bio-Rad™ Laboratories, Inc:

Table 1. Celselect Enumeration Direct Stain Kit (catalog #CEL80112).

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**
Fixing Reagent	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 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.

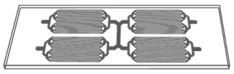

Fig. 2. Celselect Slide.

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.

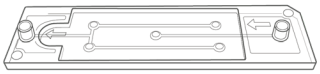

Fig. 3. Celselect Slide Manifold.

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.

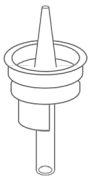

Fig. 5. Reagents Cartridge Piercer.

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

Fig. 6. Waste Jar and Tubing.

For the enumeration assays, the Genesis Priming Reservoir (Figure 7) is used during slide priming.

Fig. 7. Priming Reservoir.

Celselect Slide Reagents

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

Fixing Reagent

The Fixing Reagent enhances the rigidity of CTCs to increase probability of capture.

Reagents Cartridge

The Reagents Cartridge is shipped pre-loaded with all necessary reagents except the desired antibody solutions. These antibody solutions are added immediately before use.

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 Slide Manifold and Slide on the Genesis System.

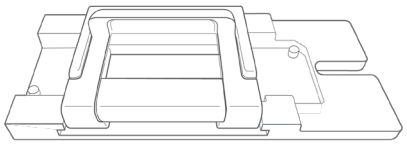

Fig. 8. Celselect Slide Station.

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

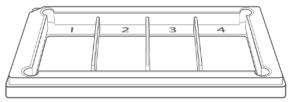

Fig. 9. Celselect Slide Imaging Adaptor.

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

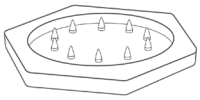

Fig. 10. Reagents Cartridge Venter.

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.

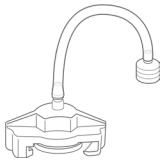

Fig. 11. Backflow Adaptor.

User-Supplied Equipment, Consumables, and Reagents

The following are additional equipment, consumables and reagents necessary to perform the CTC Enumeration Assay – Direct Staining.

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
Agilent	Lionheart LX Automated Microscope and Agilent-recommended workstation

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).

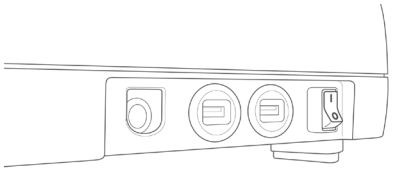

Fig. 12. Genesis System back panel.

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.

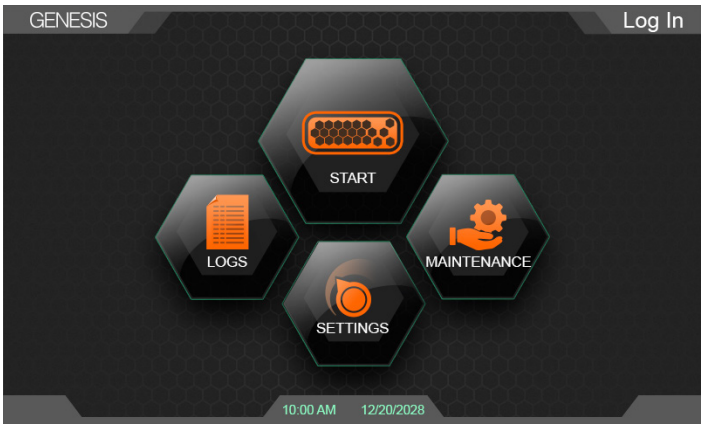

Fig. 13. Genesis GUI home screen.

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

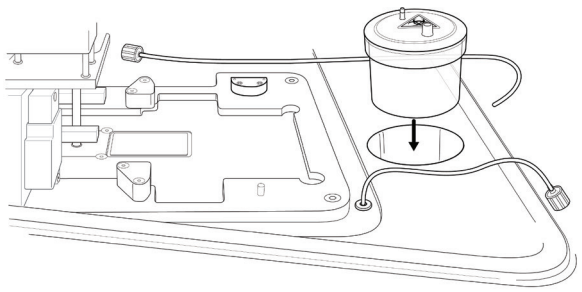

Fig. 14. Place waste jar in position.

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

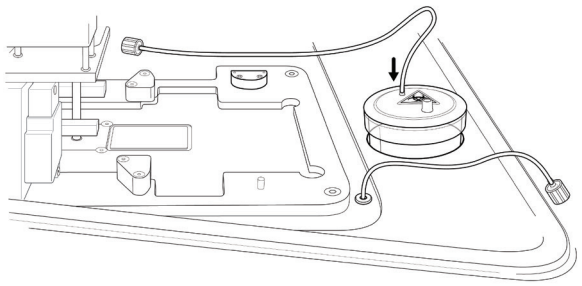

Fig. 15. Attach waste jar tubing.

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

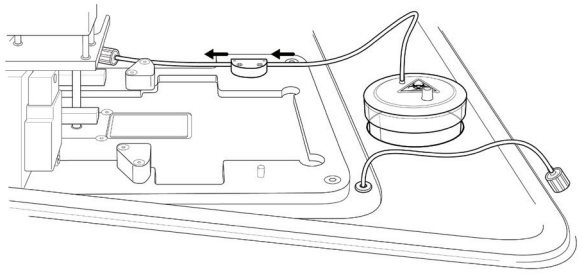

Fig. 16. Thread tubing through pinch valve.

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

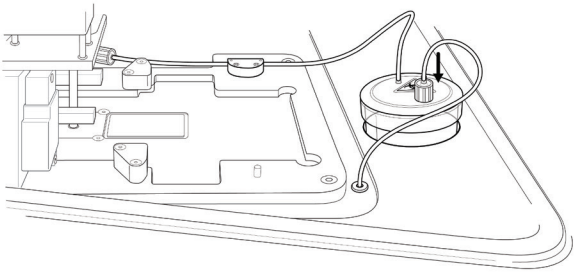

Fig. 17. Attach vacuum tube.

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

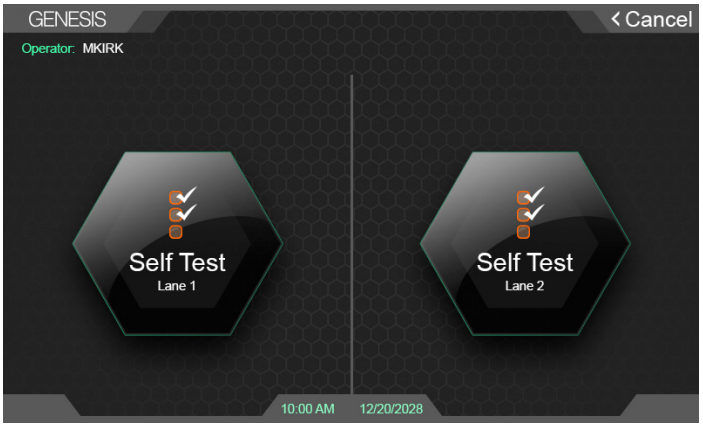

Fig. 18. Run Self Test.

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).

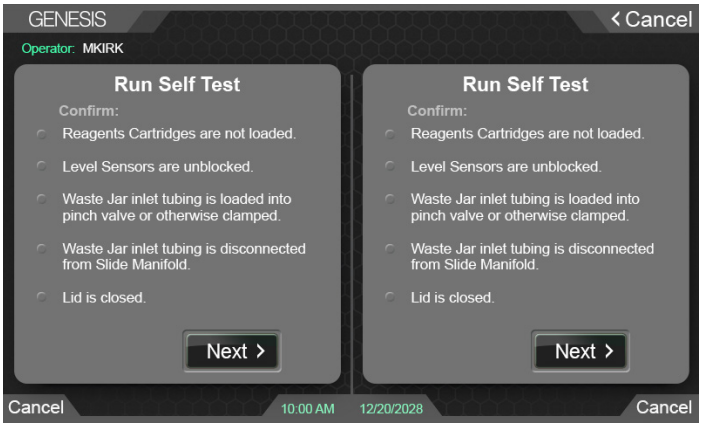

Fig. 19. Confirm Run Self Test.

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

10. When the Self Test is complete, select Enumeration - Direct Stain from the protocol drop-down list (Figure 20).

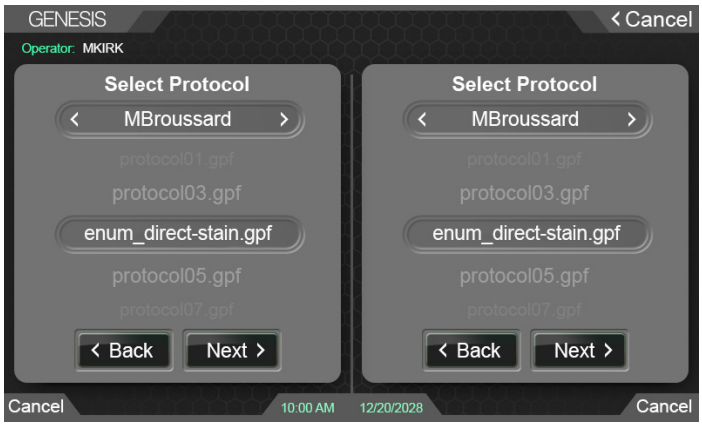

Fig. 20. After a successful Self Test, the protocols can be selected.

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

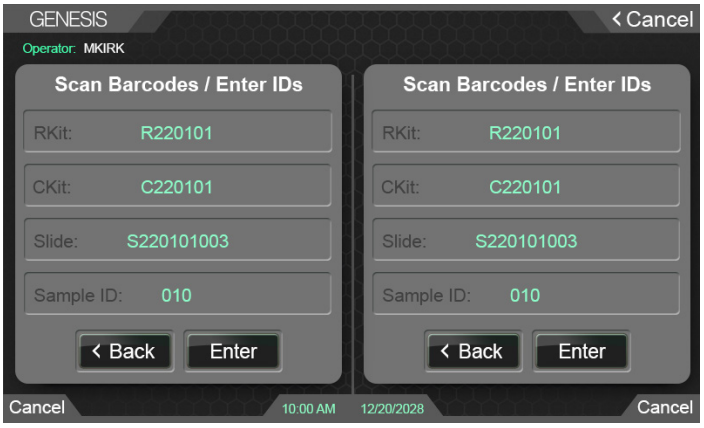

Fig. 21. Populate data for run.

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

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

b. Celselect Slides Enumeration 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

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 and to the left 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).

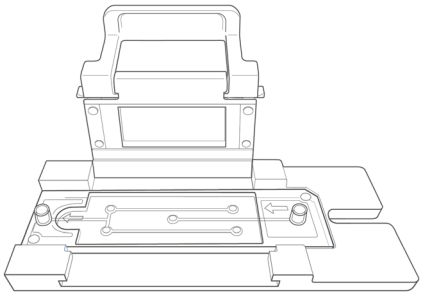

Fig. 22. Manifold loaded into the Celselect Slide Station.

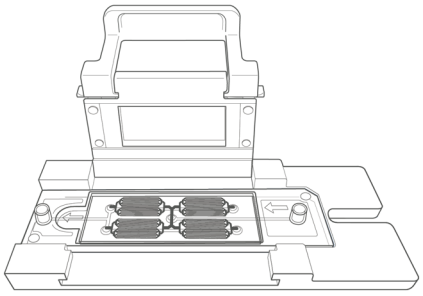

Fig. 23. Celselect Slide label-side up.

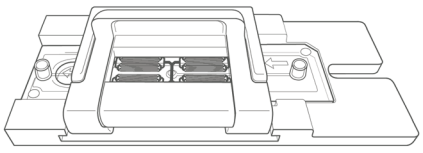

Fig. 24. Top Cover clamped on Celselect Slide and Manifold.

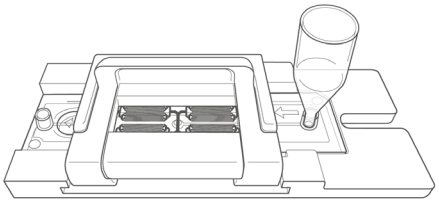

Fig. 25. Inlet Funnel in Manifold inlet port.

Priming the Celselect Slide

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 time 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).

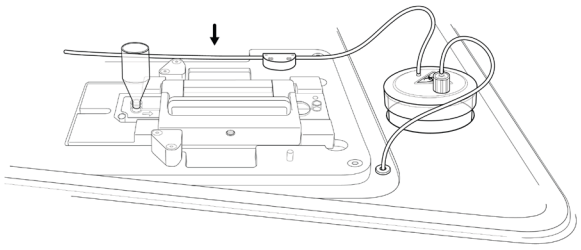

Fig. 26. Add the assembled slide station.

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

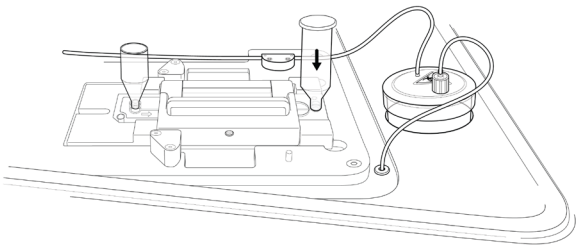

Fig. 27. Insert the Genesis Priming Reservoir.

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

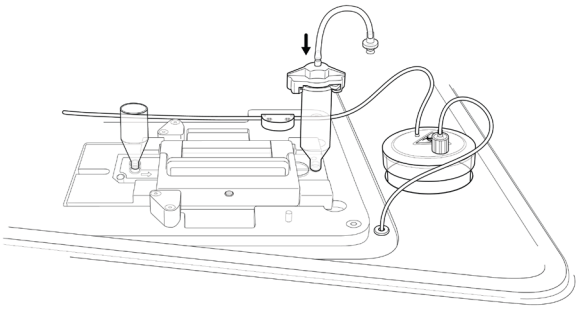

Fig. 28. Attach the Backflow Adaptor.

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

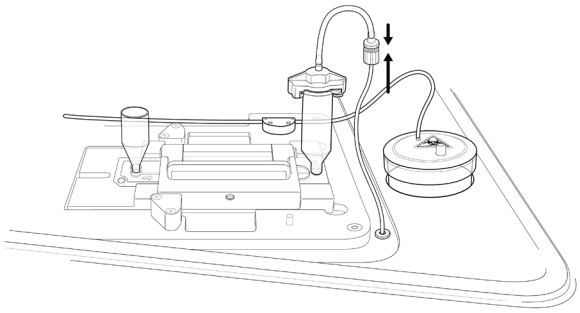

Fig. 29. Attach the vacuum tubing to the Backflow Adaptor tubing.

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 70% ethanol solution from the Genesis Priming Reservoir using a 10 mL pipette.
14. Remove the Genesis Priming Reservoir from the manifold.
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).

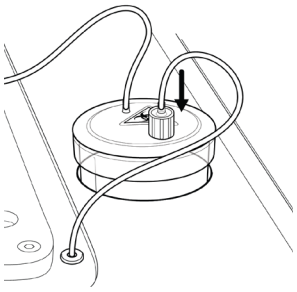

Fig. 31. Connect the vacuum tubing to the top of the waste jar.

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 and Loading Reagents Cartridge

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

1. Make an appropriate dilution of the conjugated antibodies (1 mL is required per cartridge). (For example, if making 1:200, then add 5 µL of the conjugated antibody to 1 mL of PBS.) Add additional conjugated antibodies to same solution.
2. Pierce the top foil of the Reagents Cartridge with the Reagents Cartridge Venter, making sure to twist the Venter and open the foil (Figure 32).
3. Add 1 mL of the conjugated antibody solution to chamber 8 of each Reagents Cartridge.

Note: Protect Reagents Cartridge from light and store at room temperature until use.

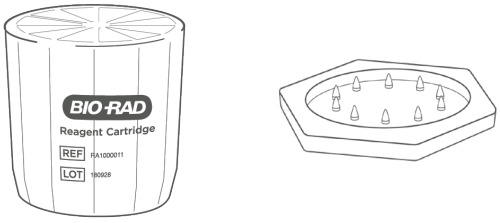

Fig. 32. Reagents Cartridge and Reagents Cartridge Venter.

Preparing the Blood Sample

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

1. Label 15 mL conical tubes for each sample.
2. For each sample, add 300 µL of Fixing Reagent to 2.7 mL of 1x PBS. Mix well.
3. 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.

4. Add 3 mL of diluted Fixing Reagent from Step 2 to the 4 mL of blood for each sample (final volume is 7 mL).
5. Incubate samples for 10 minutes on a rocker at room temperature.

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

6. Remove samples from the rocker and add 3 mL of 1X PBS (final volume 10 mL).
7. Place blood sample on the test tube rocker until use.

Note: Do not leave sample for more than 10–15 minutes before use.

Running the Genesis System

1. Add 10 mL of Sample to Inlet Funnel of each Celselect Slide Station (Figure 33).

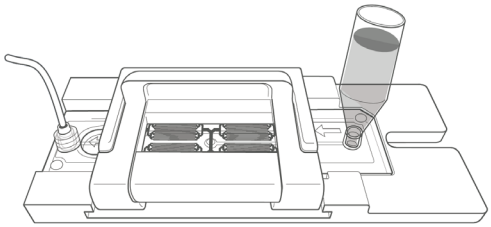

Fig. 33. 10 mL of prepared blood sample added to Inlet Funnel on Celselect Slide Station.

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

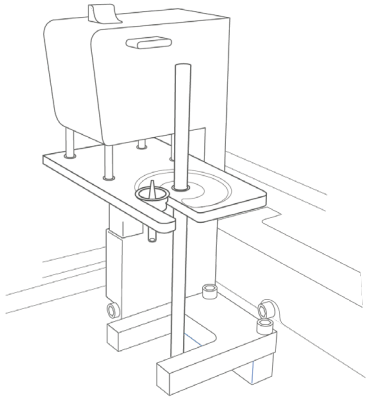

Fig. 34. Reagents Cartridge Piercer snapped in place on drip plate.

3. 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).

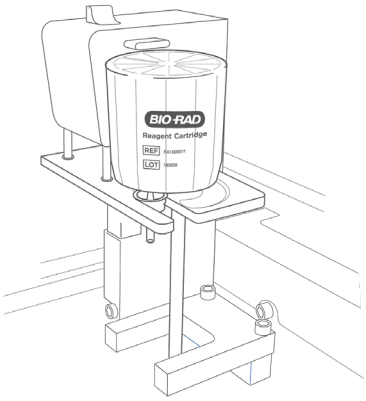

Fig. 35. Reagents Cartridge on spindle.

4. Release the cartridge retainer arm to its original position over the Reagents Cartridge.
5. Select "OK" on the User Action to initiate the rest of the run.
6. After the run is complete, select Finish. Generally, sample processing takes between 90 and 120 minutes.
7. Remove the Waste Jar tubing from the Manifold.
8. Remove the vacuum tubing from the Waste Jar.
9. Dispose of the Waste Jar and Tubing, the Reagents Cartridge, the Cartridge Piercer and the Inlet Funnel.
10. Remove the Slide Station.
11. Disassemble the Slide Station by unclamping the cover and removing the Slide and Manifold. Keep the Slide and dispose of the manifold. Cover the ports of the slide with cellophane tape and be sure to avoid wicking solution out of the slide (air decreases imaging quality).

Note: When covering the ports with cellophane tape, avoid covering the channels to limit imaging interference.

12. Store slides at 4°C until imaging. For best results process slides as soon as possible.

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 a Cell-Free DNA BCT tube (Streck, Inc.). Contact your local Streck representative for pricing and availability.

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

Table 5. Specimen Collection, Transport, and Storage.

Streck Tube Order Information	Catalog Number
Cell-Free DNA BCT 6-Tube Pack (RUO)	218961
Cell-Free DNA BCT 100-Tube box (RUO)	218962

Tubes	Transportation Temperature	Storage Temperature	Shelf Life
Streck tubes	Ship at ambient temperature. Do not put on ice.	Store at room temperature 18–30°C (65–86°F).	Process within 72 hours after collection.

B. Cell Line Spike-In Guidelines

Below is an approach to conducting a "spike-in" experiment using a CTC cell line and blood as a control for cytokeratin-positive cells or for operator training.

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 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 1.5 mL 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. Re-suspend 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. Return to Preparing the Blood Sample, Step 3.

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