Celselect Slides™ Enumeration Direct Stain Kit 2.0

User Manual

Introduction

Celselect Slides are used with the Genesis Cell Isolation System to capture and isolate individual circulating tumor cells (CTCs) based on their size, utilizing microfluidics and 140,000 individual microchambers. Isolated cells can be stained for immunofluorescence applications such as enumeration and fluorescence 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 BioTek Lionheart LX Automated Microscope (recommended). The high capture efficiency 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 Slides 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 amplify the fluorescent signal. The second approach, direct staining, utilizes a conjugated antibody that fluoresces specifically when bound to 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, see the Celselect Slides Enumeration Indirect Stain Kit 2.0 User Manual (bulletin 3338).

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; discard any clotted samples
- Protect reagents from exposure to light sources
- Do not mix and match reagents from different lots/batches
- Use protective barriers such as gloves, gowns, masks, and eye protection when working with whole blood specimens

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List of Provided Components and Consumables

Table 1 lists components and consumables included in the Celselect Slides Enumeration Direct Stain Kit 2.0.

Table 1. Celselect Slides Enumeration Direct Stain Kit 2.0 (catalog #17009841). Each kit supports processing of four samples.

Box 1 of 2. Shipment and Storage Conditions: Room Temperature	
Consumables	**Quantity**
Celselect Slides 2.0	4
Reagents cartridge piercer	4
Waste jars and tubing	4
Inlet funnel 2.0	4
Priming reservoir	4
Box 2 of 2. Shipment and Storage Conditions: 4°C	
Reagents	**Quantity**
Reagents cartridge	4

Celselect Slides and Consumables Box 1

Each of the Celselect Slides and consumables is intended for single use.

Celselect Slides (Figure 2) use Bio-Rad's proprietary CTC capture and analysis technology. Each 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 Slides 2.0.

Blood and other reagents enter the Celselect Slides through the inlet funnel 2.0 (Figure 3).

Fig. 3. Inlet funnel 2.0.

The reagents cartridge piercer (Figure 4) is firmly placed in the drip plate of the Genesis System to penetrate the bottom of the reagent cartridge and allow reagents to enter the inlet funnel.

Fig. 4. Reagents cartridge piercer.

The waste jar (Figure 5) collects reagents and biohazardous waste.

Fig. 5. Waste jar and tubing.

The Genesis System priming reservoir (Figure 6) is used during priming.

Fig. 6. Priming reservoir.

Celselect Slides Direct Enumeration Reagents Box 2

The Genesis System dispenses reagents into the funnel in single aliquots, and an entire set is provided for each run.

- **Reagents cartridge** — the reagents cartridge is shipped preloaded with all necessary reagents to facilitate cell capture and enumeration

List of Equipment Provided with the Genesis System

Table 2. Equipment provided.

Equipment	Catalog Number	Quantity
Celselect Slides Station 2.0	12020055	2
Reagents Cartridge Venter	CEL84093	1
Celselect Slides Imaging Adaptor 2.0	12019988	1
Wireless Barcode Scanner	CEL84092	1
Backflow Adaptor	12016521	2

The Celselect Slides Station 2.0 (Figure 7) is a single piece with a spring-loaded clamp to keep the Celselect Slides in place and in contact with the thermal vapor chamber beneath.

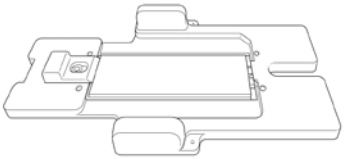

Fig. 7. Celselect Slides Station 2.0.

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The Celselect Slides Imaging Adaptor 2.0 (Figure 8) can hold up to two Celselect Slides and is designed to fit into a standard PCR plate format for use with an automated fluorescent microscope.

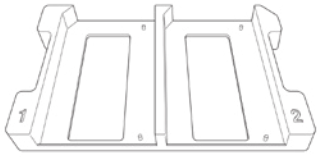

Fig. 8. Celselect Slides Imaging Adaptor 2.0.

The reagents cartridge venter (Figure 9) is designed to pierce the top foil of the reagents cartridge for its use on the Genesis System. Top-piercing is imperative to allow proper flow of reagents during sample processing.

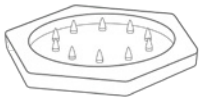

Fig. 9. Reagents cartridge venter.

The backflow adaptor (Figure 10) is designed to connect the Genesis System vacuum pump to the priming reservoir, producing positive pressure to prime slides.

Fig. 10. Backflow adaptor.

User-Supplied Equipment, Consumables, and Reagents

The equipment, consumables, and reagents listed below are necessary to perform CTC enumeration direct staining with Celselect Slides and can be purchased from any vendor.

Equipment Supplied by User

- Single-channel pipets (20, 200, and 1,000 µl)
- Serological pipet controller
- Test tube rocker
- Test tube rack
- Automated fluorescence microscope

Consumables and Reagents Supplied by User

- 5 and 15 ml conical tubes
- Sterile disposable serological pipets (5 and 10 ml)
- Phosphate buffered saline (PBS), 1x, pH 7.4
- Pipet tips (20, 200, and 1,000 µl)
- 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 11).

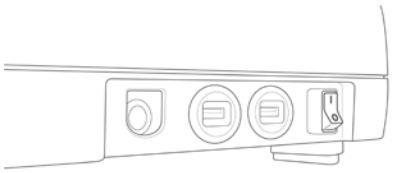

Fig. 11. Genesis System back panel.

2. Once the instrument is on, the Genesis System home screen (Figure 12) loads automatically and logs every run. From here, the user can log in, gather system information, or shut down the instrument.

Note: Log-in is not required.

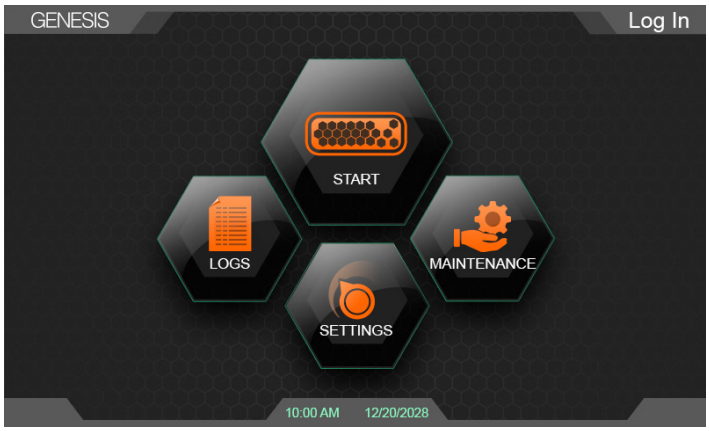

Fig 12. Genesis System home screen.

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3. Place the waste jar in position at the base of the station bays nearest the user (Figure 13).

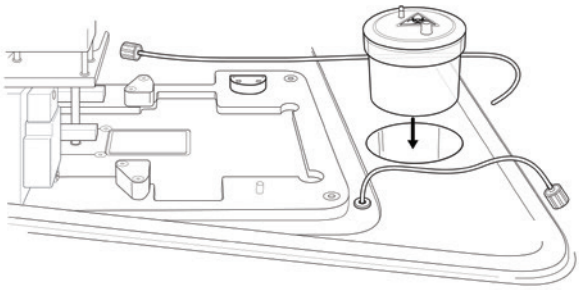

Fig. 13. Place waste jar in position.

4. Attach the waste jar tubing to the waste jar (Figure 14).

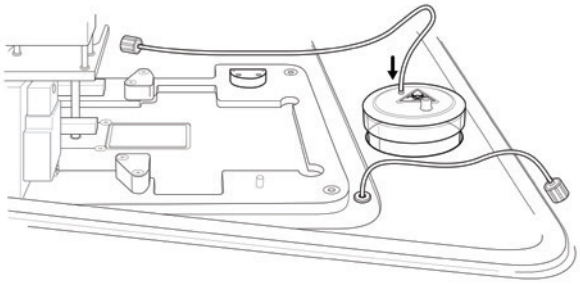

Fig. 14 . Attach waste jar tubing.

5. Press tubing into the pinch valve until you feel it click into place (Figure 15).

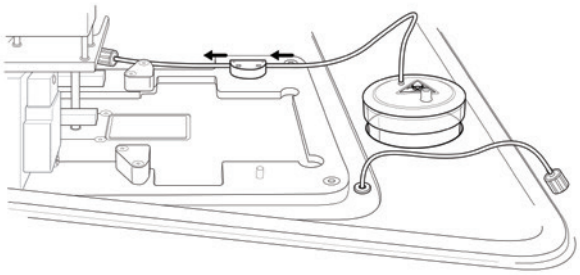

Fig. 15. Press tubing into the pinch valve.

6. Attach Genesis System tubing to the waste jar outlet (Figure 16).

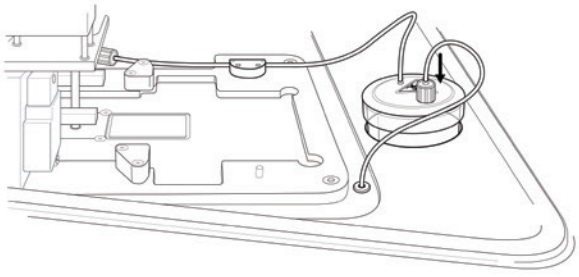

Fig. 16. Attach Genesis System tubing.

7. Press **Start** from the home screen to reach the Self Test screen. Select each lane to initiate the Self Test (Figure 17).

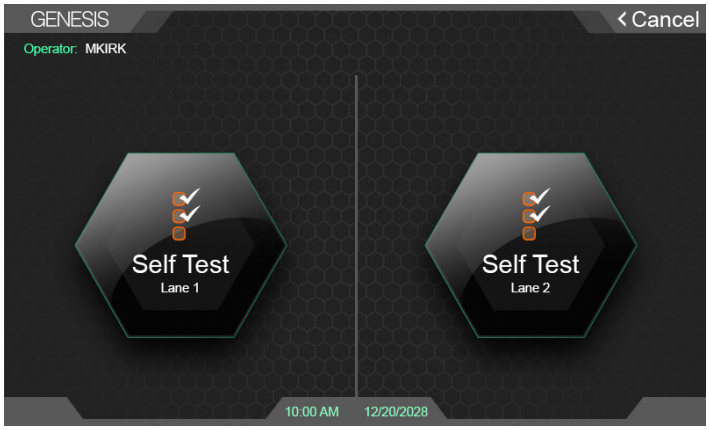

Fig. 17. Run Self Test.

8. A confirmation list will appear on the screen, which lists the actions/items the system looks for **prior** to running the Self Test (Figure 18).

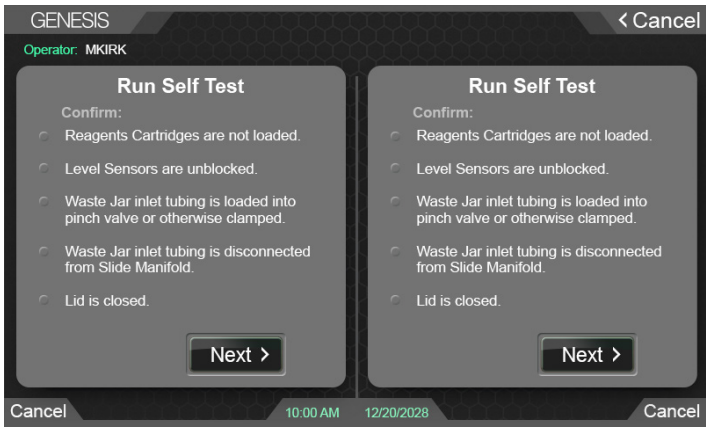

Fig. 18. Confirm Run Self Test.

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

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10. When the Self Test is complete, select **2.0 Kit Direct Enumeration** from the Select Protocol options (Figure 19).

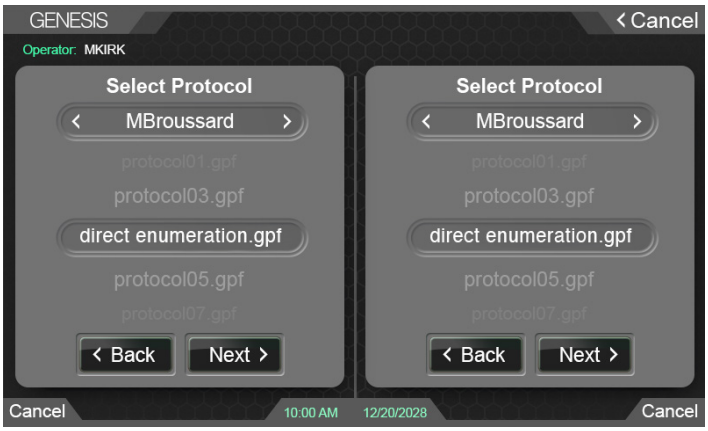

Fig. 19. After a successful Self Test, the protocols can be selected.

11. Populate the data entry fields recording the reagents, consumables, and slide identifiers using the barcode scanner (Figure 20).

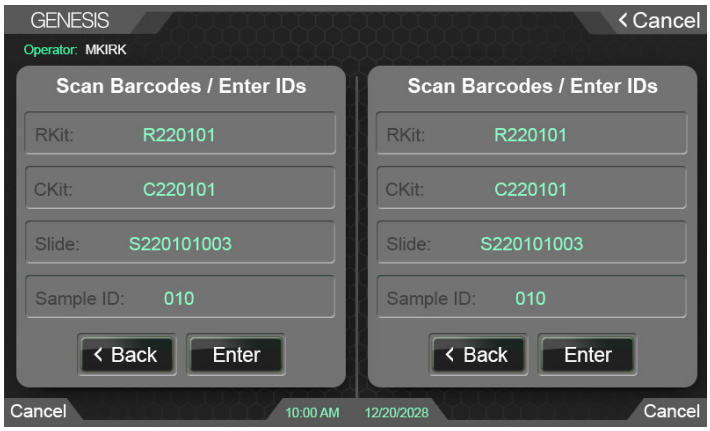

Fig. 20. Populate data for run.

Note: If you are unable to scan barcode/QR codes, use the following to manually enter the lot numbers with identifiers:

a. Celselect Slides Enumeration Direct Stain Reagents (4-pack): "C" followed by the 6-digit lot number, e.g., C010101

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 free-form field allowing the user to define a unique sample name for the run

Priming and Assembly

Note: While the slide is priming, the reagents cartridge can be pulled from 4°C storage and warmed at 37°C for a minimum of 10 minutes in an incubator or bead bath.

1. Place the slide into the slide station 2.0 by gently pulling the spring-loaded tab to allow the slide to fall into place. The notched end of the slide drops into the notched end of the slide station (Figure 21).

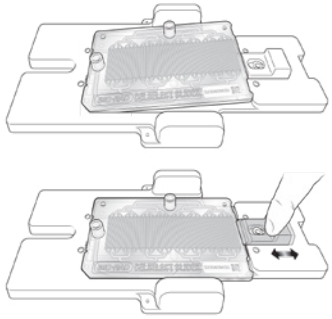

Fig. 21. Inserting the slide into the slide holder.

2. Place the slide station containing the slide on the Genesis System. Firm pressure is required to snap the slide station in place.
   - The slit in the station prevents incorrect placement
   - The slide station assembly should be pushed all the way back until its vertical arms (finger holds) meet the clamping tabs (stoppers) on the Genesis System lane (Figure 22)

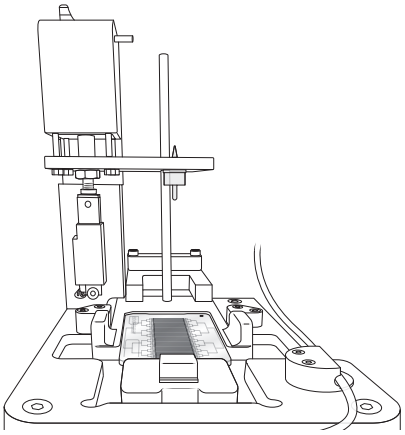

Fig. 22. Placing the slide station assembly into the Genesis System.

3. Slide the reagents cartridge piercer until it clicks or snaps into place. Do this before placing the inlet funnel. The funnel is too tall to place the piercer after placing the funnel (Figure 23).

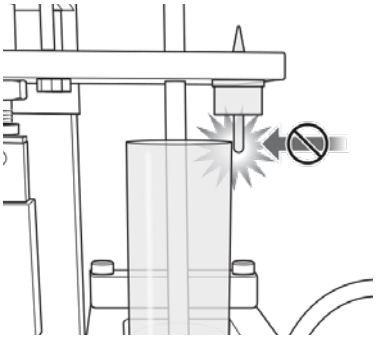

Fig. 23. Bottom piercer hitting inlet funnel if not added in correct order.

4. Tilt the inlet funnel under the piercer and attach it to the inlet on the slide.

5. Attach the backflow adaptor to the priming reservoir. Attach the reservoir to the offset inlet on the right side of the slide.

   Note: The backflow adaptor can be difficult to attach to the priming reservoir when dry. Lightly wetting the O-ring or the inside of the priming reservoir with a gloved finger or lint-free wipe saturated with either water or 70% ethanol can help the pieces slide together more easily. Using buffer is not recommended.

6. Attach the Genesis System tubing to the backflow adaptor tubing (Figure 24).

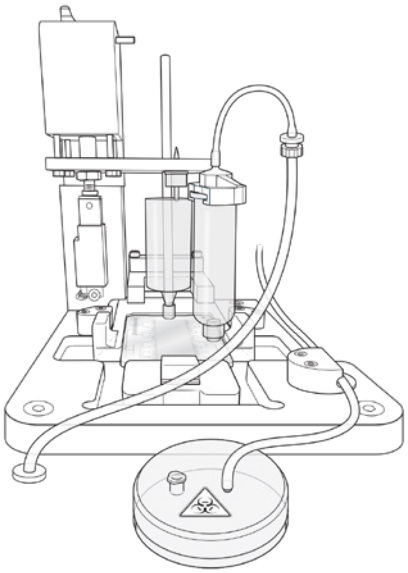

Fig. 24. Genesis System tubing attached to the backflow adaptor in preparation for slide priming.

7. Pipet 8 ml 70% ethanol into the inlet funnel and start the enrichment run on the Genesis System.

   Note: If 70% ethanol solution is prepared fresh, allow it to degas for at least 1 hour before use. Alternatively, the ethanol solution can be purchased at 70% or made ahead of time.

8. After priming, the instrument will prompt the user to remove the backflow adaptor from the priming reservoir, discard the ethanol, and replace the priming reservoir with the waste jar tubing. Connect the luer of the waste jar tubing to the slide completely by pressing down onto the outlet port. Twisting or over-tightening the luer may make it difficult to remove when the run is complete.

   Note: The ethanol will be in the priming reservoir after completion.

   Best practice: To avoid introducing air into the slide, do not remove the ethanol that remains in the outlet port.

9. Be sure to attach the Genesis System tubing to the waste jar, and press the waste jar tubing into the pinch valve (Figure 25).

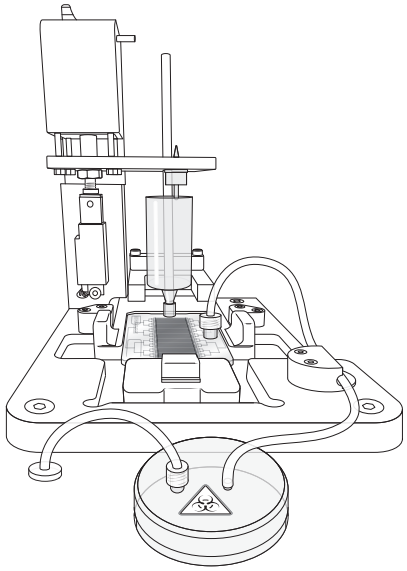

Fig. 25. Instrument loaded with slide, funnel, cartridge piercer, and waste tubing properly routed through the pinch valve.

10. Pipet 8 ml 1x PBS into the funnel and press **Resume** on the Genesis System screen to wash the slide.

11. The instrument will now prompt the user to dispense the sample and place the reagents cartridge.

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Preparing the Reagents and Loading Reagents Cartridge

Note: The reagent cartridges must to be warmed at 37°C for 10 min prior to use and left to sit 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 to open the foil.
3. Add 1 ml of the conjugated antibody solution to chamber 8 of each reagents cartridge.

   Note: Protect reagents cartridges from light and store at room temperature until use.

Sample Preparation and Processing

These steps can be done while the slide is priming. Slide priming takes about 20 minutes to complete.

1. If the biospecimen sample (blood) was kept chilled, allow the sample to acclimate to room temperature.
2. Gently mix the biospecimen sample (blood) with a serological pipet several times. Draw up to 10 ml of sample per slide into the pipet and dispense into a conical tube.

   Note: The sample will be diluted, so the conical tube must be large enough to accommodate the final volume. For one sample, a 15 ml conical tube would suffice.
3. Dilute the biospecimen sample 1:1 with room-temperature 1x PBS. For example, 7.5 ml blood + 7.5 ml 1x PBS.
4. Gently mix the sample by pipetting up and down several times with a serological pipet.
5. Before dispensing the diluted sample into the funnel, gently mix the sample by either inverting the tube several times or using a serological pipet.
6. Gently dispense the sample into the funnel and avoid generating bubbles. If large bubbles occur, they can be removed with a pipet.
7. **Important:** Pierce the top of the reagent cartridge with the reagent cartridge venter before placing it onto the Genesis System spindle.

   Note: The cartridge has one lane labeled **Start**. This should face the user.
8. Gently rotate the cartridge until it slides down the spindle, taking care not to manually twist the spindle itself.
9. Press **Resume** on the Genesis System screen.

Running the Genesis System

Below is a brief review of the steps for running your enumeration sample after the slide has been primed.

1. Add sample to the inlet funnel of each slide station (Figure 26).

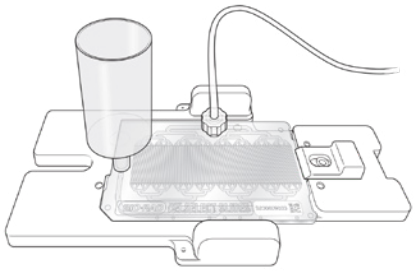

Fig. 26. Assembled slide station ready to have sample added to inlet funnel.

2. 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 27).

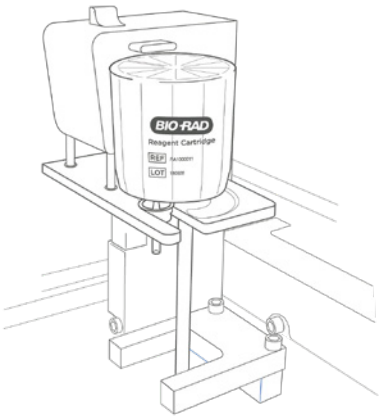

Fig. 27. Reagents cartridge on spindle.

3. Release the cartridge retainer arm to its original position over the reagents cartridge.
4. Select **OK** on the User Action to initiate the rest of the run.
5. After the run is complete, select **Finish**. Generally, sample processing takes approximately 3.5 hours for 7.5 ml of sample.
6. Remove the waste jar tubing from the slide.
7. Remove the vacuum tubing from the waste jar.
8. Dispose of the waste jar and tubing, the reagents cartridge, the cartridge piercer, and the inlet funnel.
9. Remove the slide from the slide station.

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10. 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.

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

Cleaning the Genesis System

Clean the Genesis System after each use by wiping the instrument deck with either 10% bleach solution or 70% ethanol on a towelette.

Cleaning the Slide Stations

Clean the slide stations after each use by wiping them with either 10% bleach solution or 70% ethanol on a towelette.

Disposal of Kit Consumables and Reagents

All used kit consumables and reagents should be treated as biohazardous waste and disposed of in accordance with appropriate internal laboratory safety and environmental guidelines.

Appendix

Specimen Collection, Transport, and Storage

Collect blood aseptically into EDTA tubes or Streck tubes (streck.com; see Table 3 for ordering information).

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

Table 3. Specimen collection, transport, and storage.

Streck Tube Order Information	Catalog Number
Cell-Free DNA BCT RUO, 6-tube pack	218961
Cell-Free DNA BCT RUO, 100-tube box	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
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

Cell Line Spike-In Guidelines

Below is an experiment designed to train the user on conducting the enumeration protocol. It is a spike-in experiment using a CTC line and blood.

1. Wash a flask of MCF-7 cells with 1x PBS (example: 3 ml for a T25 flask).
2. Add 0.25% trypsin to the flask (example: 3 ml for a T25 flask) and incubate at 37°C for 2–5 minutes or until cells detach.
3. Add an equal volume of media to neutralize trypsin or other dissociation reagent.
4. Transfer cell suspension solution to a 15 ml conical tube.
5. Centrifuge the suspension for 3 min at 300 rcf or 4 min at 400 rcf. Discard media and replace with fresh prewarmed media.
6. Transfer 10 µl of cell suspension to a 1.5 ml centrifuge tube.
7. Add 10 µl of Trypan Blue to the centrifuge tube and mix with cell suspension.
8. Use 10 µl of the Trypan Blue/cell mixture to count cells on a hemocytometer.
9. Prepare a dilution of 10,000 cells/ml in 1x PBS.
10. Add 50 µl of suspended cells to 4 ml of a whole blood sample.
11. Add 4 ml of PBS and gently mix by inverting the tube several times.
12. Continue by returning to step 1 of the Running the Genesis System section.

Typical Staining Patterns If Using Cytokeratin, CD45, and DAPI

Table 4. Staining patterns.

Cell Type	Antibody Stains
CTCs	CK+, DAPI+, CD45–
Leukocytes	CK–, DAPI+, CD45+

Cytokeratin is a protein expressed in the epithelium and other cohesive cells. It is commonly recognized as a CTC marker and is generally not expressed on leukocytes. DAPI is a stain for nucleic acids and is not expressed in anuclear cells such as erythrocytes. CD45, otherwise known as protein tyrosine phosphatase or leukocyte common antigen, is a surface antigen expressed by most white blood cells. It is generally not expressed by cells outside hematopoietic populations.

Antibodies are used in this panel to identify circulating cells that may be sourced from a tumor with epithelial origins. These antibodies may also be used to identify cells typically found in circulation to exclude them from analysis.

The open nature of the Genesis System allows these antibodies to be substituted or complemented for different panels of antibodies, assuming they have similar incubation settings and that appropriate changes to downstream analysis are made.

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