Element Biosciences

Cytos™ Optical Pooled Screening

User Guide

FOR USE WITH

AVITI24™ System
AVITI™ Operating Software v4.1 or later
Cytos Optical Pooled Screening Kit

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Overview

A Cytos cell screening run configured for Optical Pooled Screening (OPS) with the Cytos Optical Pooled Screening Kit uses Direct In Sample Sequencing (DISS) chemistry on the AVITI24 System to link cellular morphology with guide RNA (gRNA) identity, enabling CRISPR screens that pair each cell's phenotype with its gRNA.

The workflow requires prepared cell samples and Cytos consumables, and supports screening of up to tens of thousands of gRNAs in a single run. OPS runs typically complete in under 24 hours and include onboard secondary analysis — assigning guide RNA to cells when a Target Cell Assignment (TCA) Manifest is provided enabling gRNA identification.

Cytos OPS assay can be combined with optional protein detection using immunofluorescence-based chemistry with the Cytos Protein Cartridge, adding up to 20 additional protein markers using predefined Cytos protein panels or custom proteins. Adding the protein cartridge extends total run time by up to 24 hours depending on the number of protein targets used for the assay.

The Cytos OPS assay is enabled by two chemistries to detect RNA, protein, and cellular morphology:

- Direct In Sample Sequencing (DISS) uses Avidite Base Chemistry™ (ABC™) sequencing to sequence up to 50 bp RNA transcripts directly within intact cells.
- Fluorescence-based imaging chemistry for cell paint and protein detection. Cell paint uses a combination of fluorophore-linked antibodies and dyes to label cell membrane (wheat germ agglutinin), nuclear (RedDot2), mitochondrial (TOMM20), and actin (phalloidin) morphology markers used for cell segmentation and morphology feature extraction. Protein detection uses immunofluorescence-based imaging chemistry with biotinylated antibodies linked to fluorescent reporters from Element Biosciences or custom proteins. See *Cytos Protein User Guide (MA-00095)*.

A Cytos assay is performed using a single cartridge or multi-cartridge workflow as follows:

- **Single Cartridge** workflow uses only one cartridge type, either protein or RNA, and the run is considered complete once that single cartridge workflow is done.
- **Multi-Cartridge** workflow uses both cartridge types in sequence, protein cartridge first, followed by RNA cartridge, and the run is considered complete only after both cartridge workflows are done.

This guide includes preparing reagents, assembling the flow cell, and performing an OPS cell screening run. Before initiating a run, make sure you have read the instrument overview and safety information in the *AVITI24 System User Guide (MA-00051)*.

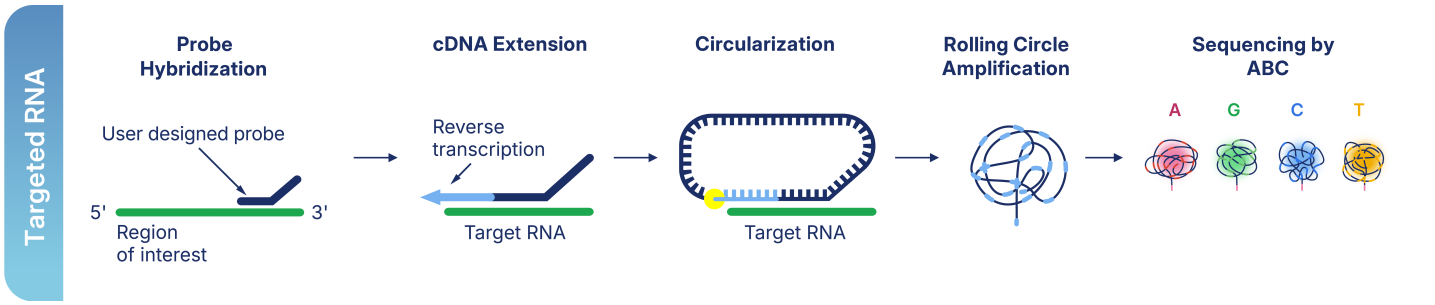
The Cytos end-to-end workflow from sample preparation to performing protein detection with a protein cartridge is documented in different guides. See *Experimental Planning* on page 9.

Direct In Sample Sequencing

A cell screening run with Direct In Sample Sequencing (DISS) on AVITI24 System with Cytos allows sequencing of nucleic acids directly within a biological sample without any library preparation steps. DISS preserves the spatial and molecular context and integrates with multiomic readouts.

Cytos workflow enables direct sequencing of targeted RNA using a single-sided probe design. The targeted sequencing chemistry utilizes a probe that hybridizes to a specific transcript or region of interest. After hybridization, reverse transcription extends the primer to generate a cDNA copy of the target RNA sequence. The cDNA is circularized and then amplified by rolling circle amplification (RCA) to form a polony for sequencing. The polony is read using ABC sequencing and the output of the Cytos assay is the sequence of the gene of interest.

The following diagram illustrates direct sequencing of targeted RNA.



Cell Screening Run

The AVITI Operating Software (AVITI OS) generates a recipe based on the assay and run parameters entered during run setup. The recipe governs each stage of the cell screening run. The run is complete when the recipe is executed and onboard analysis is complete.

A cell screening run for RNA detection can be performed **with or without** protein detection.

- **Morphology**—Uses a combination of reversible dyes and organelle probes coupled to fluorescent reporters and the fluorescence signal is imaged to detect cellular features.
- **Protein**—Uses custom or preassembled protein probes composed of biotinylated antibodies coupled to fluorescent reporters. Fluorescent signal is imaged to detect protein expression and localization.
- **Amplification**—Uses reverse transcription (RT) primers for targeted sequencing that bind to RNA transcripts which are extended into cDNA, circularized, and then amplified using rolling circle amplification (RCA) to form polonies.
- **Batches**—Each modality (morphology, protein, RNA) is processed in one or more batches, with imaging for morphology and protein detection, and sequencing for RNA detection. This approach avoids optical crowding by limiting simultaneous detection.

OPS Run

OPS Run with Protein

Cell Screening Run Consumables

An OPS run on the AVITI24 System requires a Cytos Optical Pooled Screen Cartridge and buffer bottle, a slide kit, Cytos Guide RNA Control Primers, and flow cell assembly kit. To perform a dual flow cell run, a quantity of two of each kit is required.

For a list of kits with catalog numbers, see *Consumables and Tools* on page 35.

Cytos OPS Cartridge

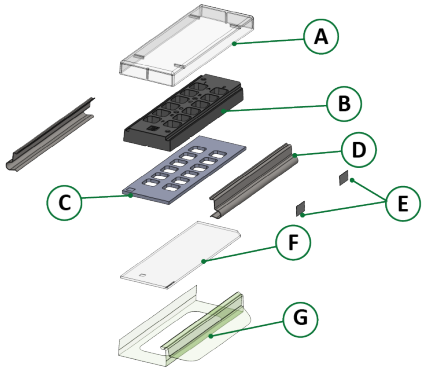
The Cytos OPS cartridge provides reagents for a Cytos OPS run with DISS to enable the co-detection of morphology and RNA in a single run. The cartridge allows addition of custom primers required for an OPS run.

Custom primers for the Cytos OPS cartridge are designed in ElemBio Cloud Custom Designer™. See the ElemBio™ Cloud Online Help. After designing the primers, you can order from a third-party vendor. Custom primers are added to the Cytos OPS cartridge before the run. See *Add Primers to the Cartridge* on page 31.

NOTE
Protein co-detection with Cytos OPS assay uses a multi-cartridge workflow. Run the Cytos Protein Cartridge workflow **first** using the Cytos Protein Kit. See *Cytos Protein User Guide (MA-00095)*. After the protein workflow is complete, the instrument run is paused to allow you to review the protein data and load the Cytos OPS cartridge. Follow instructions in this guide to set up the Cytos OPS cartridge workflow. See *Run Setup for Multi-Cartridge Workflow* on page 30.

Cytos Slide Kit

The Cytos Slide Kit is used for culturing cell samples onto the slide. The slide kit includes a glass slide with a barcode for tracking and validation, a frame, a gasket, two side clips, pull tab, and a lid. The frame and the gasket determine the number of wells on the slide. Before starting a Cytos run, the slide is reassembled as a flow cell with parts from the AVITI24 Flow Cell Assembly Kit.

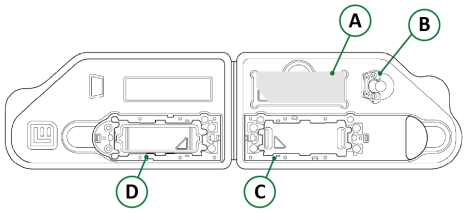


A Lid
B Frame
C Gasket
D Side clip
E Sticker tab
F Glass slide
G Pull tab

Slide kits are available in 48-well, 12-well, or 1-well configurations with uncoated surfaces. Uncoated slide kits need to be coated with a custom surface specific to your cell line before use in cell culture. See Custom Surface Coatings chapter in the *Cytos Sample Preparation & Optimization User Guide (MA-00094)*.

AVITI24 Flow Cell Assembly Kit

The AVITI24 Flow Cell Assembly Kit contains an adhesive slide, two flow cell gaskets, and the top and bottom frame. Before the run, the sample slide is assembled and packaged into a Cytos flow cell using the provided components. Based on the slide kit that you are using, select the appropriate AVITI24 Flow Cell Assembly Kit. See *Assemble the Cytos Flow Cell* on page 19.

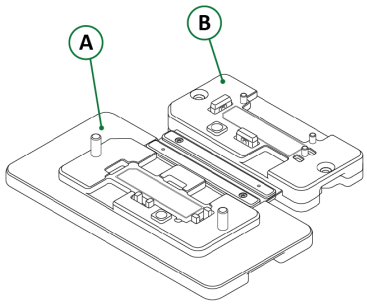


A Adhesive slide
B Flow cell gaskets (2)
C Frame bottom
D Frame top

Flow Cell Assembly Tool Set

The Flow Cell Assembly Tool Set includes tools to convert the Cytos Slide Kit into a flow cell using parts provided in the AVITI24 Flow Cell Assembly Kit.

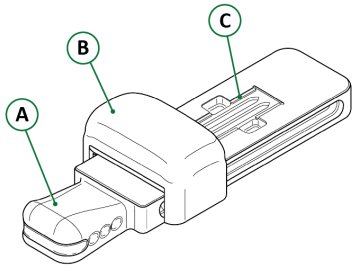
- **Flow Cell Aligner**—The flow cell aligner aligns and adheres the sample slide from the slide kit to the adhesive slide provided in the AVITI24 Flow Cell Assembly Kit. See *Align and Seal the Slides* on page 20.



A Side labeled **Sample** for sample slide

B Side labeled **Adhesive** for adhesive slide

- **Flow Cell Sealer**—The flow cell sealer ensures a secure seal of the two affixed slides as you *slowly* move the roller grip forward and back. For care instructions, see *Maintain the Flow Cell Sealer* on page 37.



A Base grip

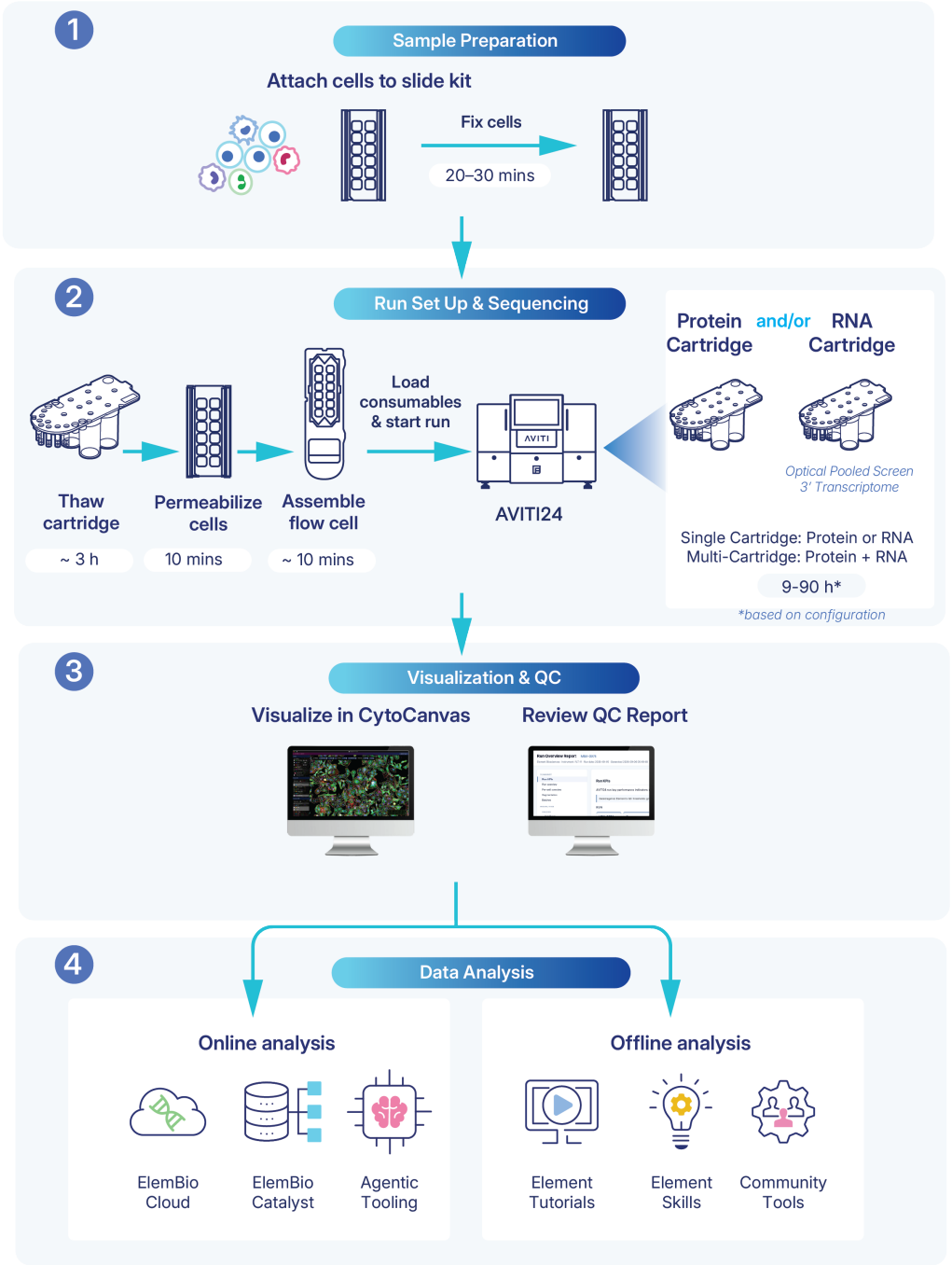
B Roller grip

C Indentation for slides

Experimental Overview and Planning

This section provides an overview of the Cytos end-to-end workflow, list of required materials, and provides references to additional user guides needed to complete the workflow.

Experimental Overview



Experimental Planning

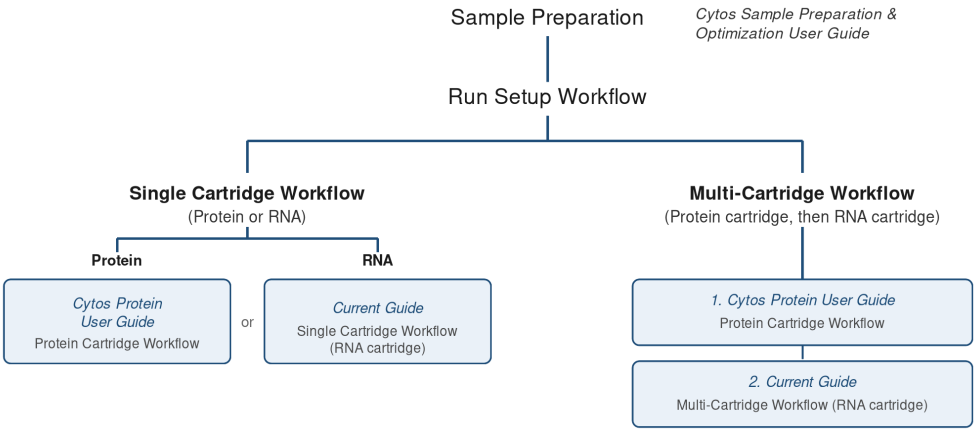
This guide provides instructions for performing an OPS run using the Cytos Optical Pooled Screening Kit with samples prepared on Cytos Slide Kits. Use this guide together with the applicable supporting guides referenced below to complete sample preparation and, if applicable, protein detection.

For sample preparation, see *Cytos Sample Preparation & Optimization User Guide (MA-00094)*.

Depending on your experimental design, follow one of these workflows:

- **Single Cartridge Workflow**: For RNA detection without protein detection, use the RNA single cartridge workflow in this guide.
- **Multi-Cartridge Workflow**: For RNA detection following protein detection, **first** complete the protein cartridge workflow using the *Cytos Protein User Guide (MA-00095)* and then return to this guide to complete the RNA multi-cartridge workflow.

The following diagram shows the applicable guide for each stage of the workflows:



The following materials are required for performing an OPS run. For details and storage conditions, see *Consumables and Tools* on page 35. For details and supplier information on materials supplied by the user, see *User-Supplied Consumables* on page 36.

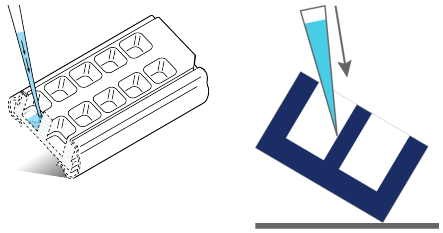
Materials	Included with a Kit / User Supplied
Cytos Slide Kit with fixed cells	See *Cytos Sample Preparation & Optimization User Guide (MA-00094)*
For Cytos Protein assay (if your assay includes protein detection)	See *Cytos Protein User Guide (MA-00095)*
For OPS assay	
Cytos Optical Pooled Screening Kit	Included with the kit
Two custom primers per target	Design using ElemBio Cloud Custom Designer User Supplied
AVITI24 Flow Cell Assembly Kit	Available for purchase
Flow Cell Assembly Tool Set (includes flow cell sealer and flow cell aligner)	Available for purchase
Pipettes (16-channel for 48-well slide kits or 8-channel for 12-well slide kits)	User Supplied
Ethanol (EtOH), biological grade	User Supplied
Phosphate-buffered saline (PBS), 1X, pH 7–7.4	User Supplied
Biological-grade/RNase-free water or 10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA (Low TE buffer)	User Supplied
Isopropyl alcohol (IPA) wipes, lens wipes, and lint-free Kimwipes	User Supplied

Sample Preparation

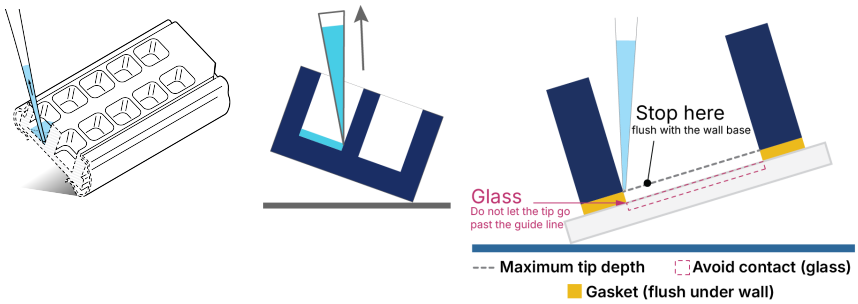
The Cytos Optical Pooled Screening Kit workflow requires cell samples prepared on Cytos Slide Kits and fixed with a fixation reagent (formaldehyde). See *Cytos Sample Preparation & Optimization User Guide (MA-00094)* for detailed sample preparation protocols and guidance.

If the samples were stored or shipped, ensure the slide kit was not damaged during storage and shipping. Examine the cells with bright field microscopy to ensure there is no cell loss after the fixation step.

Follow these pipetting guidelines during on-flow cell permeabilization protocol:



- When adding liquid, slowly dispense along the wall of the well.
- Tilt the slide kit such that you can see the bottom corner of the well.
- Dispense slowly to reduce force of the liquid onto the slide surface.
- Do not contact the slide surface.



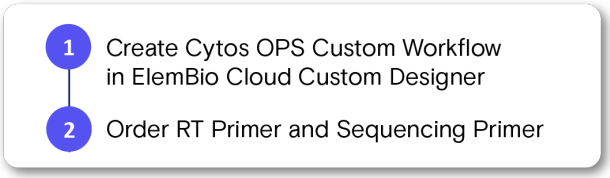
- When removing liquid, position the pipette tip in the corner of the well, resting against the gasket.
- Tilt the well while pipetting such that the tip enters the well at an angle.
- Do not contact the slide surface. Do not allow the tip to go past the gasket. Touching the glass with the pipette tip can damage the surface.

Design Custom Workflow

A Cytos OPS assay requires two custom primers per target designed using ElemBio Cloud Custom Designer: the reverse transcription (RT) primer and the sequencing primer. After the compatible oligo sequences are generated, order the primers from a third-party oligo vendor. See Custom Primer Design in Online Help.

The Cytos OPS assay can be performed **with or without protein** detection depending on the experimental goals. Use the ElemBio Cloud Custom Designer to design your specific workflow configuration.

Protocol Summary



Design the OPS Custom Workflow

1. Go to the **ElemBio Cloud Custom Designer**.

   If you are not signed in to ElemBio Cloud, you might need to enter your organization name, user name, and password to access ElemBio Cloud Custom Designer.
2. Enter the design identification details.
3. Follow the onscreen instructions to configure your Cytos workflow based on your experimental goals.

   All configurations include 4-plex cell paint for morphology readouts (cell membrane, nucleus, actin, and mitochondria).

   » Select RNA and then Guide RNA for OPS.

   » [Optional] Select Protein if you plan to include protein detection and then choose up to a maximum of 20 protein targets in the Protein Module. Select from a choice of available Cytos protein panels, or a full custom protein panel, or a combination of custom proteins with Cytos protein panels.

   » In the Guide RNA Sequencing module, define sequencing target sites to generate compatible oligo sequences for the RT primer and sequencing primer. Create a TCA manifest.

   NOTE
   ElemBio Cloud Custom Designer provides the RT primer and sequencing primer sequence that is most compatible to your target site.
4. Order the RT primer (must be 5′ phosphorylated) and sequencing primer with the specified sequences from a third-party oligo vendor for CRISPR analysis.
5. [Optional] Order Cytos protein panels. See *Cytos Protein Panels* on page 35.

   To prepare custom protein probes, see *Cytos Protein User Guide (MA-00095)*.

After publishing the custom design, a design package with details is generated that can be printed or shared with collaborators. The custom workflow is available for selection in a planned run or the AVITI24 manual run setup form for instruments with access to ElemBio Cloud. For offline instruments, download the design package and load the zipped folder onto the AVITI24 at run setup.

Run Preparation

Run preparation includes thawing the Cytos cartridge and adding appropriate reagents, if applicable.

Prepare cell samples on a Cytos Slide Kit in advance of run preparation and setup. See *Cytos Sample Preparation & Optimization User Guide (MA-00094)*. Additionally, ensure you have designed and ordered custom primers required for OPS.

NOTE
If you are performing a multi-cartridge run, ensure the protein cartridge workflow is complete and you have reviewed the protein summary data (charts and thumbnails) before thawing the RNA cartridge.

Protocol Summary

Run Preparation

1. Thaw reagent cartridge
2. Thaw custom and control primers

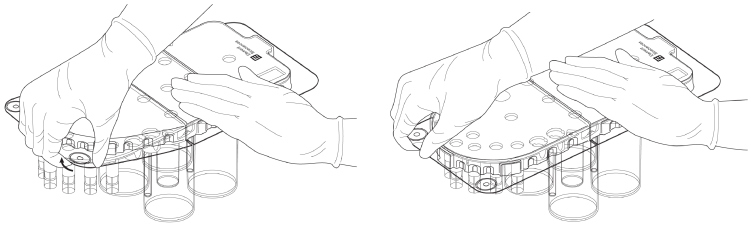
Thaw Reagent Cartridge

1. Remove a cartridge from -25°C to -15°C storage.

   CAUTION
   Protect the cartridge from light. The cartridge contains light-sensitive reagents.

2. Remove the shipping cover:

   a. While supporting the cartridge, lift the removal tab at the left corner until it releases from the cartridge.



   b. Moving across the front edge of the shipping cover, repeatedly lift the edge until the cover is fully released.
   c. Pull to remove the remainder of the shipping cover from the cartridge.

3. Place the cartridge in a room-temperature water bath and thaw for ~3 hours. Do not submerge the foil seals.
4. Inspect each well to make sure all reagents are fully thawed. Reagents thaw at varying rates.
   If ice remains in any well, return the cartridge to the water bath until fully thawed.
5. If not immediately initiating the run, place the thawed cartridge at 2°C to 8°C and keep away from light. **Do not exceed 3 hours.**

Thaw Primers

1. When the reagent cartridge is almost thawed, remove the custom RT primer, custom sequencing primer, and Cytos Guide RNA Control Primers from -25°C to -15°C storage.
2. Thaw custom RT primer and sequencing primer at room temperature for 15 minutes. Gently invert each tube several times to mix. Set aside on ice.
3. Thaw Cytos Guide RNA Control Primers (RT Control Primer and Seq Control Primer) at room temperature for 15 minutes. Set aside on ice.

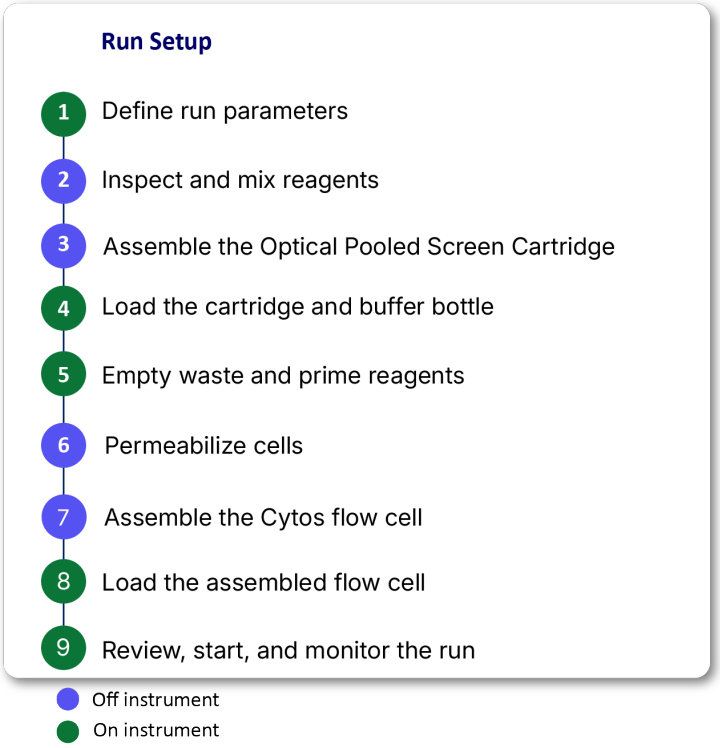
Run Setup for Single Cartridge Workflow

> If your assay includes protein detection, follow the run set up for multi-cartridge. See *Run Setup for Multi-Cartridge Workflow* on page 30.

Performing a cell screening run on an AVITI24 System includes steps to permeabilize cells, load consumables, assemble the flow cell, and then follow prompts on the AVITI OS interface to set up the run.

Before initiating a run, review the overview, software, troubleshooting, and safety information in the user guide for your instrument.

Protocol Summary



Set Up a Cell Screening Run

1. Stage run manifests for import:
   - » If setting up the run manually, save manifests on a USB and connect the USB drive to an instrument USB port.
   - » Alternatively, you can save manifests to the specified Server Message Block (SMB) storage connection.
   - » If you planned the run in ElemBio Cloud, upload manifests to the planned run.

   NOTE
   For runs that include Target Cell Assignment, two manifests are required. One is the Run Manifest, which provides cell type information for segmentation. The second is the Target Cell Assignment Manifest, which assigns target regions to cells. See TCA manifest in Online Help for details.
2. On the Home screen, select **New Run**.
3. For run type, select **Cell Screening**.

4. Select a side or both sides to use for the run.
   - » **Side A**—Set up a run on side A.
   - » **Both**—Set up simultaneous runs on sides A and B.
   - » **Side B**—Set up a run on side B.
5. Select **Next** and proceed to one of the following steps:
   - » For a **Manual Run**, proceed to *Define Manual Run Parameters*.
   - » For a **Planned Run**, proceed to *Select a Planned Run*.

Define Manual Run Parameters

1. Make sure **Manual Run** is selected for the type of run.
2. In the Run Name field, enter a unique name to identify the run.
   The field accepts 1–64 alphanumeric characters, hyphens (-), and underscores (_).
3. [Optional] In the Run Manifest field, select **Browse** and import a run manifest.
   You can import a run manifest from an inserted USB drive or from an SMB storage connection.
4. In the Storage drop-down menu, select a storage location or leave the default selection.
5. [Optional] In the Description field, enter a description that represents the run.
   The field accepts ≤ 500 alphanumeric characters, hyphens, underscores, spaces, and periods (.).
6. In the Well Layout field, select **48**, **12**, or **1** well.
7. Select **No** for Cell Culture Optimization Run.
8. In the Custom Workflow drop-down menu, go to **OPS**.
   a. Select the custom workflow for the run as follows:
      - For instruments with access to ElemBio Cloud, select the name from the workflow drop-down menu.
      - Otherwise, select **Upload a Workflow Definition Package**, navigate to an inserted USB drive or storage location, and select the file.
   b. Select the **Target Cell Assignment manifest** from the TCA Manifest drop-down menu.
9. Under **Advanced Settings**:
   - » If you are using a custom recipe, select **Browse** and import the custom recipe file from a USB drive.
   - » For the *Save Raw Imaging Projections* option, the default setting is **Yes**.
     This saves original cell paint image projections captured from the flow cell before image processing, normalization, background correction, or other processing is applied. Adds 50–100 GB per run output.
   - » For the *Save per Target Background Images* option, the default setting is **No**.
     Select **Yes** to track background intensity over time and enable background subtraction. Saving per-target background images increases final data output size and run time as images are saved for every imaging target.
   - » For the *Illumination Correction* option, the default setting is **Yes**.
     Illumination correction estimates the extracellular background signal to simultaneously subtract extracellular background signal and correct for variability in illumination across the field of view.
10. Select **Next** to proceed to prepare reagents or setup Run Side B screen.
11. If applicable, repeat steps 2–10 to setup side B.
12. Proceed to *Inspect and Mix Reagents*, next page.

Select a Planned Run

1. Select **Planned Run**.

   AVITI OS displays a list of compatible planned runs for the instrument and run type.

   For information on planned run compatibility, see *Run Planning* in the Online Help.
2. Select the run you want to use from the list of planned runs.
3. Review the run parameter fields to make sure they are correct.

   If you need to edit a planned run, modify it in ElemBio Cloud. See *Edit a Planned Run* in the Online Help.
4. In the Storage drop-down menu, select the storage connection for the run.
5. Select **Next** to proceed to the Prepare Reagents or the Run Side B screen.
   - » After you proceed, the selected planned run becomes unavailable for other connected instruments.
   - » If you exit run setup before priming, the run returns to the list of available planned runs.
6. If applicable, repeat steps **2**–**5** to set up a dual start run with a second planned run.

Inspect and Mix Reagents

1. Inspect each cartridge well to make sure reagents are fully thawed.
2. Gently invert the cartridge *10 times* to mix reagents.

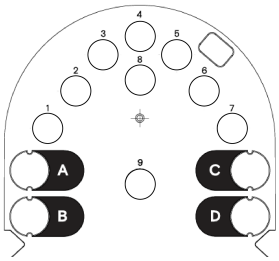
   CAUTION
   Inadequately mixed reagents can cause run failure.
3. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.
4. Inspect the small tubes to make sure all liquid is at the bottom of the tube.
5. Place the cartridge into a clean cartridge basket and lock the clips. Wipe any excess moisture.

Dilute Custom Primers

1. Dilute each custom sequencing primer to 100 µM using nuclease-free water or Low TE buffer (10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA). Set aside on ice.
2. Dilute each custom RT primer to 100 µM using nuclease-free water or Low TE buffer. Set aside on ice.
3. Further dilute each of the RT primer to 4 µM using nuclease-free water or Low TE buffer. Set aside on ice.

Add Primers to the Cartridge

Custom RT primer and sequencing primer are required for a Cytos OPS cartridge in positions C and D.



Position **C**—RNA RT Primer
Position **D**—RNA Sequencing Primer

1. Using a clean pipette tip, pierce the foil seal of the RNA RT Primer tube in position **C** of the cartridge.
2. Add 24 µl of each custom RT primer (4 µM) to the RNA RT Primer tube. Gently pipette 15 times to mix.

   Use the RT primer pool on the same day.

3. Using a clean pipette tip, pierce the foil seal of the RNA Seq Primer tube in position **D** of the cartridge.
4. Add 24 µl of each custom sequencing primer (100 µM) to the RNA Seq Primer tube. Gently pipette 15 times to mix.
5. Add Cytos Guide RNA Control Primers:
   - » Using a clean pipette tip, add 24 µl RT Control Primer into RNA RT Primer tube in position **C** of the cartridge. Using a P-1000 pipette, gently pipette 15 times to ensure thorough mixing.
   - » Using a clean pipette tip, add 24 µl Seq Control Primer into the RNA Seq Primer tube in position **D** of the cartridge. Using a P-1000 pipette, gently pipette 15 times to ensure thorough mixing.

Confirm Reagent Preparation

1. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
2. Select the **Insert into basket** checkbox to confirm that the cartridge is in the cartridge basket.
3. For OPS cartridge, make sure the RT primers and sequencing primers are present on the cartridge.
4. Confirm each of the consumable preparation steps displayed on the screen and select the checkbox.
5. Select **Next** to proceed to the Load Reagents screen.

Load Cartridge and Buffer

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Slide the basket containing the thawed cartridge into the reagent bay until it stops.
4. Support the buffer bottle with both hands and slide it into the reagent bay until it stops.
5. Close the reagent bay door, and then select **Next** to proceed.

Empty Waste and Prime Reagents

1. Open the waste bay door.
2. Unscrew the transport cap from the cap holder above the waste bay.
3. Remove the waste bottle from the waste bay and close the transport cap.

   CAUTION
   Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional regulations.

4. Open the transport cap and the vent cap.
5. Support the waste bottle with both hands and empty the waste:
   a. Position the bottle over the funnel or waste receptacle.
   b. Tip the bottle forward and drain. Invert the bottle and shake to expel all droplets.
   c. If necessary, wipe liquid off the bottle.
6. Close the vent cap and return the empty waste bottle to the waste bay.
7. Screw the transport cap onto the cap holder and close the waste bay door.

   NOTE
   Before priming, if you need to discard the run setup, click **Discard** on the top left side of the screen, correct the run setup, and reuse the same cartridge or add a new cartridge as needed. Proceed to the priming step.

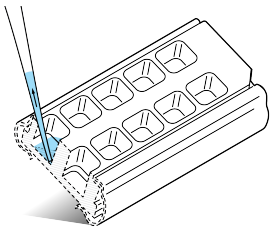
8. Select **Next** to *automatically* start priming. Priming takes approximately 27 minutes.
9. While priming is progressing, proceed to *Permeabilize Cells* on page 18.

Permeabilize Cells

For permeabilization, use ethanol and do not exceed a 10-minute incubation. Minimize the time between permeabilization and starting the run. Do not allow the wells to dry during liquid exchange steps.

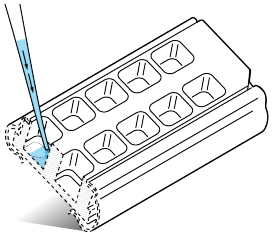
NOTE
See *Sample Preparation* on page 10 for pipetting guidelines. See Knowledge Base for detailed videos on pipetting guidance.

1. Prepare permeabilization reagent (70% ethanol)—Prepare **fresh** daily.
   - » 7 ml ethanol (EtOH), biological grade
   - » 3 ml biological-grade/RNase-free water
2. Retrieve the slide kit containing fixed cells.
3. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.



4. Slowly add the appropriate volume of 1X PBS along the middle of each well wall to wash the wells once with 1X PBS.

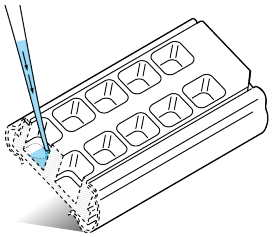
Reagent	48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
1X PBS	50 µl	200 µl	2 ml



NOTE
If you notice bubbles in the 48-well slide kit, place the 48-well slide kit in a Cytos Centrifuge Adapter and centrifuge at 130 x g for 2.5 minutes. Balance the centrifuge with another slide kit tray.

5. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
6. Slightly tip the slide kit and slowly add the appropriate volume of permeabilization reagent along the middle of each well wall.

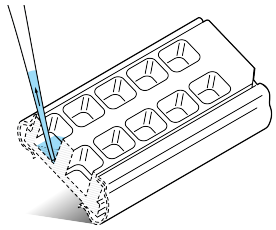
Reagent	48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
Permeabilization reagent	50 µl	150 µl	1.5 ml



7. Cover the slide kit and incubate for **10 minutes at room temperature**. Do not exceed a 10-minute incubation.

8. To wash the wells, slightly tip the slide kit and remove the appropriate volume of permeabilization reagent from each well. Then, slowly add the appropriate volume of 1X PBS along the middle of each well wall.

Reagent	48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
1X PBS	50 µl	200 µl	3 ml



9. Repeat the wash **three more times**. Do not remove the liquid after the final wash.
10. Leave the cells in 1X PBS. Proceed to *Assemble the Cytos Flow Cell*.

Assemble the Cytos Flow Cell

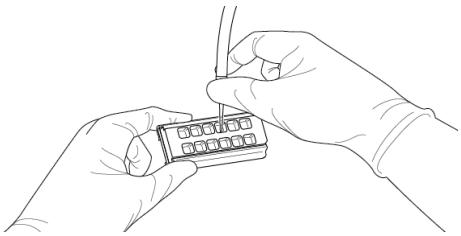
To assemble the Cytos flow cell, gather the following tools, kits, and recommended equipment:

- Vacuum aspiration system with 200 µl tip (recommended)
- Flow cell aligner and flow cell sealer
- AVITI24 Flow Cell Assembly Kit

> **IMPORTANT**
> For best results, load the flow cell within 10 minutes of assembly. Do not allow the flow cell to dry.

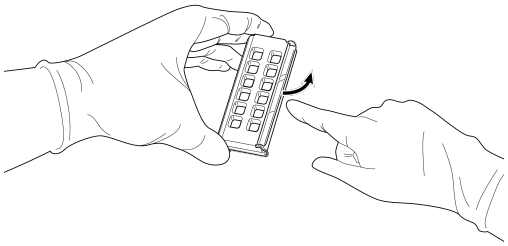
Disassemble the Slide Kit

1. Wear a clean pair of gloves before disassembling the slide kit.
2. Use a vacuum aspiration system with a 200 µl tip to remove the liquid from each well of the slide kit:
   a. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
   b. Make sure no flowing liquid is observed.

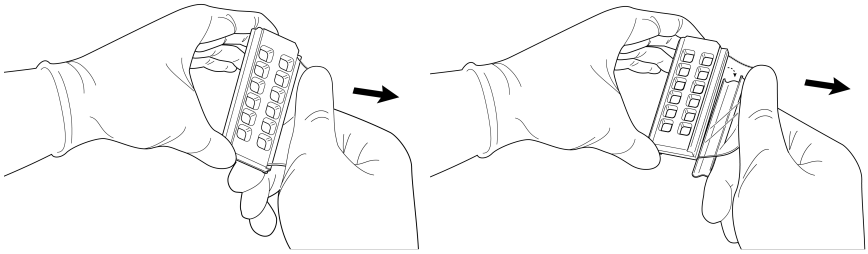


3. Hold the slide kit over the bench so clips do not fall to the ground during disassembly. Hold one side of the slide kit along the metal clip. Take care to avoid placing any pressure on the slide.

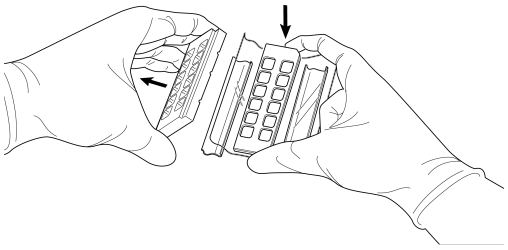
   See the video on slide kit disassembly.
4. To remove the metal side clips, locate the plastic pull tab on the side of the slide kit and grip the pull tab to remove it from the adhesive.



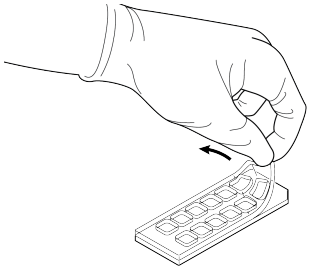
5. Grab the pull tab flap and pull the flap outwards with a firm force to dislodge the metal clip closer to the pull tab. Apply even force and avoid pulling from one spot of the pull tab.



6. Once the first metal clip is dislodged, lift the top-right beveled corner of the gasket to allow some air between the gasket and the frame. Then, firmly lift the frame from the gasket. The second metal clip is easily dislodged. Do not use the pull tab to remove the second metal clip.



7. Lift the glass slide with the gasket and remove the plastic pull tab underneath the glass slide. Discard the pull tab.
8. Grip the top-right corner of the slide kit gasket and gently pull to remove it from the sample slide.

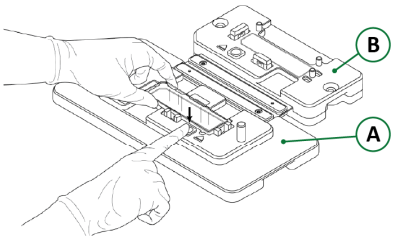


Align and Seal the Slides

1. Make sure the surface of the flow cell aligner is clean. Thoroughly wipe both sides of the aligner with ethanol, including all pins.

> **CAUTION**
> **Avoid damage to the slide.** Handle slides with care to avoid breakage. Follow instructions to avoid chipping of the edges.

2. Wear a clean pair of gloves before handling the slide. Do not write directly on the slide.
   See Knowledge Base for detailed videos on aligning and sealing the slides.
3. Load the sample slide onto the flow cell aligner:
   a. Press and hold the button on the **Sample** side of the flow cell aligner.
   b. Align the beveled corner of the sample slide with the beveled corner markings on the aligner.
   c. Make sure the sample slide is well-seated in the recessed area and release the button.

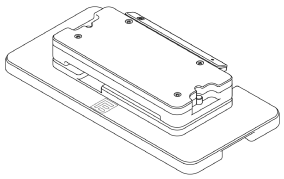


A **Sample** side
B **Adhesive** side

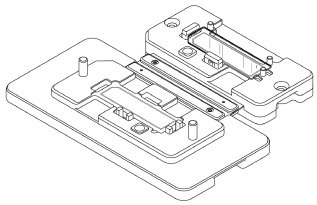
4. Open the AVITI24 Flow Cell Assembly Kit and remove the adhesive slide from the package.

   CAUTION
   Handle the slide from the edges only.

5. Load the adhesive slide onto the flow cell aligner:
   a. Press and hold the button on the **Adhesive** side of the flow cell aligner.
   b. Align the beveled corner of the adhesive slide with the beveled corner markings on the aligner.
   c. Make sure the adhesive slide is well-seated in the recessed area and release the button.
6. Starting from the beveled corner, peel off the protective easy-peel film from the adhesive slide.
7. Close the aligner to affix the sample slide and adhesive slide:
   a. Using two hands, one on each side, lift and fold the **Adhesive** side of the flow cell aligner over the **Sample** side.
   b. Align the posts on the **Sample** side with the holes on the Adhesive side.
   c. Guiding the Adhesive side with both hands, slowly allow the **Adhesive** side to make contact with the **Sample** side.



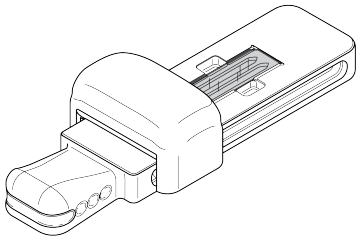
8. Gently place your hand on the aligner for 5 seconds. Excessive pressure can damage the slides.
9. With both hands, lift the **Adhesive** side to open the flow cell aligner. The sample slide is affixed to the adhesive slide.



10. Clean the surface of the flow cell sealer with an isopropyl alcohol wipe. Thoroughly wipe the recessed slide holder.
    For more information, see *Maintain the Flow Cell Sealer* on page 37.

NOTE
Incomplete or improper cleaning of the flow cell sealer can introduce blemishes on the slide that may not be visible to the eye but can appear as artifacts in imaging results.

11. Position the flow cell sealer on a flat surface so the roller grip moves forward and back in front of you, not side to side.
12. Place the aligned slides on the flow cell sealer in the recessed slide holder. Make sure the slides are well-seated.



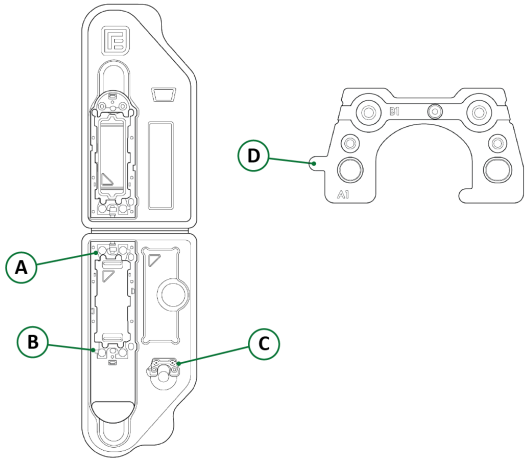
13. Hold the flow cell sealer roller grip with one hand and the base handle with the other hand. *Slowly* move the roller grip forward and then back, taking ~2 seconds to roll in each direction. With each roll, make sure the slides remain well-seated in the recessed area. Repeat the forward and back movement 2 times.

NOTE
Moving the roller grip slowly ensures a proper seal and avoids damage to the slides.

14. Flip over the aligned slides and place in the recessed slide area of the flow cell sealer. Repeat step **13** an additional 3 times.

Assemble the Flow Cell Cassette

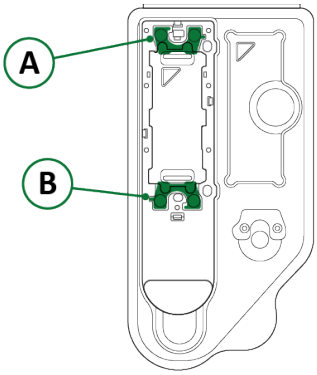
1. Open the AVITI24 Flow Cell Assembly package and locate the two gaskets stored in the package.



A Gasket location above slide area
B Gasket location below slide area
C Gaskets stored in flow cell assembly package
D Gasket key

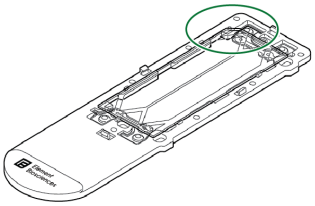
2. Position each of the two flow cell gaskets onto the bottom frame of the flow cell cassette, one above and one below the slide area. Make sure the gasket key is properly seated in the recess.

CAUTION
Gaskets *must* be present to prevent run failure.



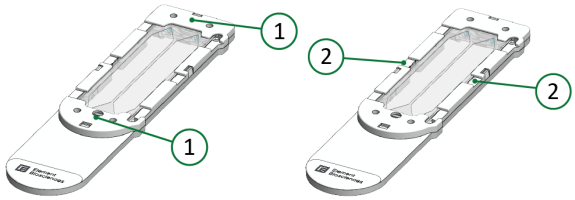
A Gasket placed above slide area
B Gasket placed below slide area

3. Remove the slides from the flow cell sealer.
4. Place the sealed slides onto the bottom frame of the flow cell cassette with the beveled corner in the top-left position as shown on the packaging. The cassette design ensures only one orientation. Make sure the slides rest flat on the bottom frame.



5. Align the top frame of the flow cell cassette over the bottom frame.

6. Press down on the cassette in four places to secure the top frame to the bottom frame until you hear a click.
   a. First, press down near the top and bottom of the slide area.
   b. Second, press down on each side of the slide area.



7. Visually inspect the flow cell cassette to make sure there are no gaps along the sides. If a gap is visible, repeat step **6** to ensure the top and bottom frames are fully engaged.

Clean the Assembled Flow Cell

1. **Wear a clean pair of gloves before handling the assembled flow cell.**
2. Wipe the assembled flow cell surface with an isopropyl alcohol wipe.
3. Immediately dry the surface with a lens wipe to prevent isopropyl alcohol from drying and leaving any streaks on the flow cell that can interfere with onboard imaging.

   NOTE
   If visible droplets or streaks are observed, repeat steps 1 and 2.
4. [Optional] Holding the flow cell firmly, use canned air to ensure the flow cell is free of dust.

   Be sure to hold the canned air in an upright position and the air nozzle is not too close to the flow cell to minimize blowing any contaminants onto the surface which can cause imaging defects.
5. Proceed immediately to *Load the Flow Cell*.

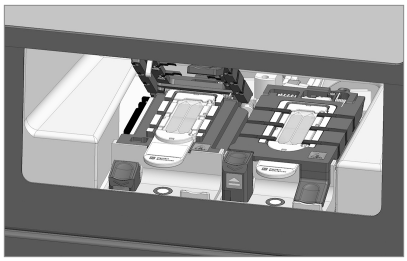
Load the Flow Cell

IMPORTANT
Handle the nest carefully during each interaction to avoid any scratches and defects. Pulling the flow cell out forcefully or at an angle can scratch the nest surface.

1. To proceed to the Load Flow Cell screen, select **Next** on the AVITI OS interface after priming is complete.

   AVITI OS moves the nest forward and opens the nest bay door. A brief delay is normal.
2. Make sure the nest status light is blue.
3. Press the button to the left of the nest to open the lid. Make sure to fully press down on the button.

   —Failure to fully press down on the button can cause errors when closing the lid or aligning the flow cell.—
4. Remove the used flow cell from the nest.
5. Before wiping the nest, inspect the nest carefully to ensure there is no visible debris. If any debris is visible, gently remove and discard the debris.
   Wipe the nest with a lens wipe dampened with isopropyl alcohol and allow to dry. If necessary, use a polyurethane foam-tip swab to clean additional areas around the nest.
6. With the label facing up, place the assembled flow cell over the three registration pins on the nest.



7. Guide the flow cell slightly to the left of the nest as you lower the tab on the right side of the lid until the lid snaps into place. The flow cell shifts a bit to the right.

   —The nest status light turns green.—

8. Select **Close Nest** to close the nest bay door and retract the stage.
9. Select **Next** to *automatically* start the Flow Cell Integrity Test.

   If the Flow Cell Integrity Test fails, you can recover the flow cell and save the run. See *Flow Cell Recovery* on page 28.

10. After the Flow Cell Integrity test successfully completes, select **Next**.

Review and Start the Run

1. On the Details page, review the run parameters:

Parameter	Description
Cartridge	The cartridge type
No. Wells	The number of wells on the flow cell
Storage	The location where run output is stored
Manifest	The file name of the uploaded run manifest, if applicable
Description	A description of the run (optional)
Advanced	If applicable, advanced run settings for the run, such as custom recipe
Custom Design	Lists custom design details

NOTE
If you notice any errors in run set up, see the guidance in Knowledge Base to discard the run, skip the priming step, and restart the run setup process.

2. Select Consumable Information to review the flow cell, cartridge, and buffer bottle information:

Field	Description
Lot Number	The manufacturing batch number assigned to the consumable
Expires on	The date that the cartridge and buffer bottle expires
Serial Number	The unique identifier for the consumable or all zeros for an unscanned barcode
Part Number	The part identifier for the consumable

   —A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.—

3. Select **Run** to start the run.
4. Process the materials removed from the reagent bay:
   » If you removed a used cartridge and buffer bottle, follow the instructions in *Discard the Cartridge and Bottle* on page 27.

Monitor Run Metrics

1. Select **Overview** or **Details** to toggle between views of run details.
2. Monitor run metrics as they appear onscreen. AVITI OS indicates the expected batch during which metrics appear.

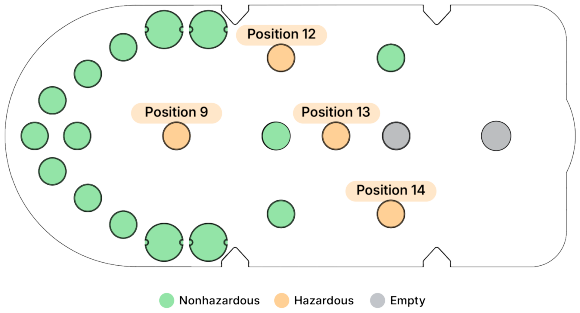
—Expected cycles are approximate, and all metrics are estimates.—

3. Continue monitoring the run as AVITI OS refreshes the metrics.
4. When the run is complete, leave all materials on the instrument.
   - » To return to the Details view, select **Overview**.
   - » To access run data, go to your storage location.

Discard the Cartridge and Bottle

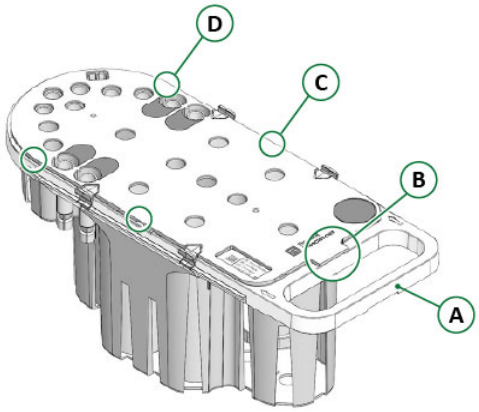
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources.

The following wells contain hazardous reagents. The position numbers in the figure align with the position numbers in the SDS.



Dispose of Reagents

1. Keep the cartridge in the basket with the clips locked.
2. Hold the basket handle with one hand and lift the cartridge lid tab with the other. Expect resistance at three points.



A Basket handle
B Cartridge lid tab and first point of resistance
C Second point of resistance on both sides of the cartridge
D Final point of resistance on both sides of the cartridge

3. Remove the hazardous wells from the cartridge.
   —The volume remaining in each well depends on the number of cycles performed.—
4. Using a pipette tip or a similar tool, enlarge the hole in each foil seal to form a triangle.

5. Empty each well into hazardous waste or other appropriate container per the SDS.
6. Unlock the clips and remove the cartridge from the basket.
7. Remove the remaining wells from the cartridge and enlarge the hole in each foil seal.
8. Empty each well into the appropriate container per the SDS.
9. Discard the cartridge and buffer bottle per the SDS.
10. Rinse the basket with water and dry upside down.

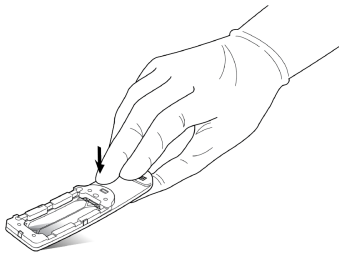
Flow Cell Recovery

If the Flow Cell Integrity Test fails during run setup, perform the following steps to recover the flow cell.

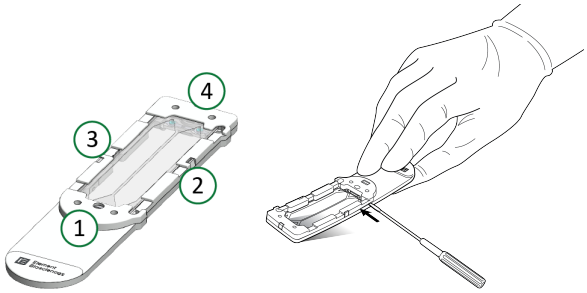
IMPORTANT
Ensure careful handling each time nest interaction is instructed. Handle the nest delicately during any interaction to avoid scratches and defects. Pulling the flow cell out forcefully or at an angle can scratch the nest surface.

1. Open the nest and remove the flow cell.
2. Use a vacuum aspiration system or pipette at the end ports to remove all liquid from the flow cell. Make sure the flow cell is completely dry.
3. Release each of the four snap positions that secure the top half of the cassette to the bottom half in the order listed:

   a. With the flow cell on a flat surface, lift the cassette handle upward with light pressure to slightly bend the cassette and create a gap between the top half and the bottom half.

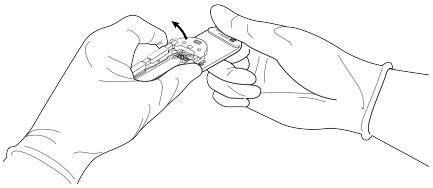


   b. Starting with position 1 below the slide area, insert a flat and rigid tool, such as a small screwdriver, into the gap along the side of the cassette and toward the snap location. Maintain pressure on the cassette and gently rotate the tool to release the snap.



   c. With position 1 released, insert the tool at positions 2 and 3 along the side of the cassette. Gently rotate the tool to release each snap.

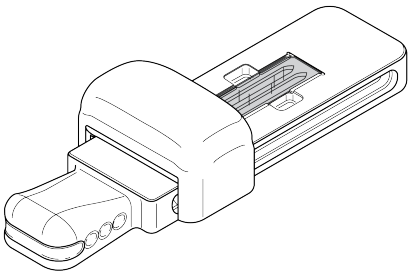
   d. To release the snap at position 4, rotate the top half of the cassette upward or use the tool from the side of the cassette.



4. Lift to remove the slides from the bottom half of the flow cell cassette.
5. Re-seal the slides using the flow cell sealer:

   a. Make sure the surface of the flow cell sealer is clean. Thoroughly wipe the flow cell sealer and the recessed slide holder.

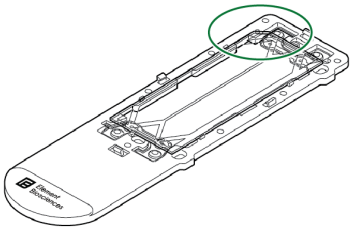
b. Place the affixed slides in the recessed slide holder. The slides must be well-seated to avoid damage to the slides.



c. Hold the flow cell sealer roller grip with one hand and the base handle with the other hand. *Very slowly* move the roller grip forward and then back, taking ~5 seconds for each pass. Repeat at least 3 times.

d. Flip over the slides and reposition in the recessed slide area of the flow cell sealer. Repeat the forward and back roller motion an additional 3 times.

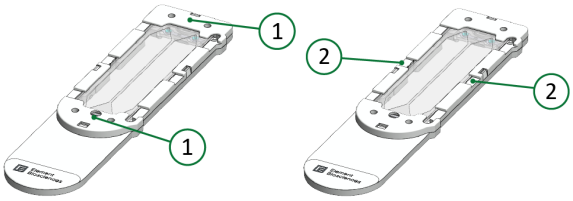
6. Visually inspect the flow cell gaskets to make sure each gasket is well-seated.
7. Place the sealed slides onto the bottom half of the flow cell cassette with the beveled corner in the top-left position. Make sure the slides rest flat on the cassette bottom.



8. Align the top half of the flow cell cassette over the bottom half.
9. Press down on the cassette in four places to secure the top half to the bottom half until you hear a click.

   a. First, press down near the top and bottom of the slide area.

   b. Second, press down on each side of the slide area.



10. Visually inspect the flow cell cassette:
    - » Make sure there is no damage to the plastic and that the flow cell cassette rests flat on a flat surface.
    - » Make sure there are no gaps along the sides of the cassette top and bottom.
11. Wipe the assembled flow cell surface with an isopropyl alcohol wipe and dry the surface with a lens wipe.

NOTE
Be sure to inspect for any streaks or smudges before loading on the instrument.

12. Reload the flow cell on the instrument and make sure the flow cell is well-seated on the nest.
    - » If the flow cell is well-seated on the nest, follow the software prompts to resume the run.
    - » If the flow cell does not seat properly on the nest or the Flow Cell Integrity Test fails again, you must cancel the run.

Run Setup for Multi-Cartridge Workflow

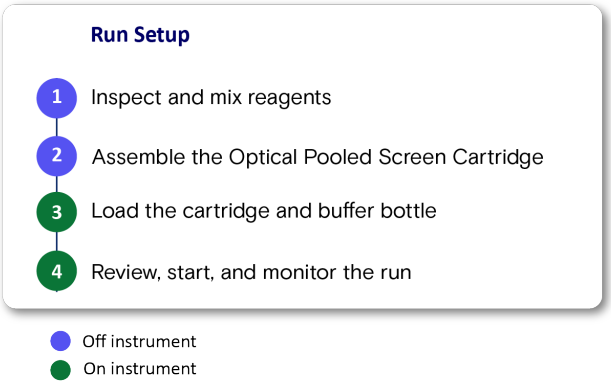
> If your assay does not include protein detection, follow the run set up for a single cartridge. See *Run Setup for Single Cartridge Workflow* on page 14.

Perform a multi-cartridge workflow after the initial Cytos Protein Cartridge workflow is complete and a quick review of the protein data summary is done to ensure the protein run meets quality control specifications. The instrument display shows the first cartridge is complete and run is paused to load the second cartridge.

IMPORTANT
Load and initiate the second cartridge workflow within the specified time displayed onscreen.
If you are unable to load the second cartridge within the specified time, the run automatically stops, onscreen display shows Stop, and you cannot load the second cartridge. The data is available only for the first protein cartridge. Before starting another run, perform a maintenance wash as described in the *AVITI24 System User Guide (MA-00051)*.

Run setup for a multi-cartridge workflow includes steps to load the second cartridge, AVITI Buffer Bottle, and then follow prompts on the AVITI OS interface to continue the run. Click on **Load Now** to begin the run setup.

Protocol Summary



Inspect and Mix Reagents

1. Inspect each cartridge well to make sure reagents are fully thawed.
2. Gently invert the cartridge *10 times* to mix reagents.

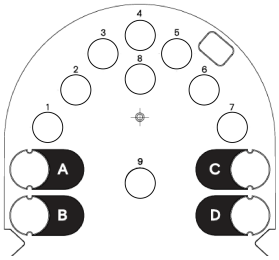
   CAUTION
   Inadequately mixed reagents can cause run failure.
3. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.
4. Inspect the small tubes to make sure all liquid is at the bottom of the tube.
5. Place the cartridge into a clean cartridge basket and lock the clips. Wipe any excess moisture.

Dilute Custom Primers

1. Dilute each custom sequencing primer to 100 µM using nuclease-free water or Low TE buffer (10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA). Set aside on ice.
2. Dilute each custom RT primer to 100 µM using nuclease-free water or Low TE buffer. Set aside on ice.
3. Further dilute each of the RT primer to 4 µM using nuclease-free water or Low TE buffer. Set aside on ice.

Add Primers to the Cartridge

Custom RT primer and sequencing primer are required for a Cytos OPS cartridge in positions C and D.



Position **C**—RNA RT Primer
Position **D**—RNA Sequencing Primer

1. Using a clean pipette tip, pierce the foil seal of the RNA RT Primer tube in position **C** of the cartridge.
2. Add 24 µl of each custom RT primer (4 µM) to the RNA RT Primer tube. Gently pipette 15 times to mix.

   Use the RT primer pool on the same day.
3. Using a clean pipette tip, pierce the foil seal of the RNA Seq Primer tube in position **D** of the cartridge.
4. Add 24 µl of each custom sequencing primer (100 µM) to the RNA Seq Primer tube. Gently pipette 15 times to mix.
5. Add Cytos Guide RNA Control Primers:
   - » Using a clean pipette tip, add 24 µl RT Control Primer into RNA RT Primer tube in position **C** of the cartridge. Using a P-1000 pipette, gently pipette 15 times to ensure thorough mixing.
   - » Using a clean pipette tip, add 24 µl Seq Control Primer into the RNA Seq Primer tube in position **D** of the cartridge. Using a P-1000 pipette, gently pipette 15 times to ensure thorough mixing.

Confirm Reagent Preparation

1. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
2. Select the **Insert into basket** checkbox to confirm that the cartridge is in the cartridge basket.
3. For OPS cartridge, make sure the RT primers and sequencing primers are present on the cartridge.
4. Confirm each of the consumable preparation steps displayed on the screen and select the checkbox.
5. Select **Next** to proceed to the Load Reagents screen.

Load Cartridge and Buffer

1. Open the reagent bay door.
2. Remove any materials from the reagent bay and set aside.
3. Slide the basket containing the thawed cartridge into the reagent bay until it stops.
4. Support the buffer bottle with both hands and slide it into the reagent bay until it stops.

   NOTE
   Be sure to use a fresh buffer bottle for the second cartridge.
5. Close the reagent bay door, and then select **Next** to proceed.

Review and Start the Run

1. On the Details page, review the run parameters:

Parameter	Description
Cartridge	The cartridge type
No. Wells	The number of wells on the flow cell
Storage	The location where run output is stored
Manifest	The file name of the uploaded run manifest, if applicable
Description	A description of the run (optional)
Advanced	If applicable, advanced run settings for the run, such as custom recipe
Custom Design	Lists custom design details

NOTE
If you notice any errors in run set up, see the guidance in Knowledge Base to discard the run, skip the priming step, and restart the run setup process.

2. Select Consumable Information to review the flow cell, cartridge, and buffer bottle information:

Field	Description
Lot Number	The manufacturing batch number assigned to the consumable
Expires on	The date that the cartridge and buffer bottle expires
Serial Number	The unique identifier for the consumable or all zeros for an unscanned barcode
Part Number	The part identifier for the consumable

—A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.—

3. Select **Run** to start the run.
4. Process the materials removed from the reagent bay:
   » If you removed a used cartridge and buffer bottle, follow the instructions in *Discard the Cartridge and Bottle* on page 27.

Monitor Run Metrics

1. Select **Overview** or **Details** to toggle between views of run details.
2. Monitor run metrics as they appear onscreen. AVITI OS indicates the expected batch during which metrics appear.

   —Expected cycles are approximate, and all metrics are estimates.—
3. Continue monitoring the run as AVITI OS refreshes the metrics.
4. When the run is complete, leave all materials on the instrument.
   » To return to the Details view, select **Overview**.
   » To access run data, go to your storage location.

Data Analysis

AVITI24 performs onboard analysis providing analysis-ready outputs that are available in ElemBio Cloud for cloud-connected instruments or in user-specified local storage for offline instruments. After a Cytos run completes, the raw data is ready for secondary and tertiary analysis, and is compatible with Element analysis packages or community platforms.

Refer to the following resources for support with AVITI24 multiomics data analysis:

- ElemBio Cloud Online Help provides a comprehensive workflow and instructions along with tutorials for analyzing cell screening data generated using the AVITI24 system. Run output files, run field descriptions, and metrics/charts are also listed here.
- QC report generated by both onboard analysis and secondary analysis to assess run performance.

Troubleshooting

The following troubleshooting information addresses problems that can occur during an OPS run using the Cytos Optical Pooled Screening Kit.

For troubleshooting issues with sample preparation or cell culture optimization, see *Cytos Sample Preparation & Optimization User Guide (MA-00094)*.

Problem	Resolution
Targets not assigned	• Ensure the correct gRNA sequences are loaded into ElemBio Cloud Custom Designer and correct RT primer and sequencing primers are ordered. See Online Help for details. • Be sure to add the RT primers and sequencing primers into the cartridge before starting the run. Follow the instructions in this guide to load the correct volume/concentration of the primers and in the specific well position in the cartridge. • Verify the Cytos Guide RNA Control Primers are present which serve as a chemistry control.
Low assignment rate	• Ensure the correct concentration and volume of sequencing primers are added to the cartridge as described in this guide. • Use cells and construct at the recommended expression level. • Ensure cells have undergone proper selection. Follow published protocols on cell selection for your cell type. • Review TCA manifest and confirm the targets are listed in the correct orientation. See guidance in Online Help to input the correct information in the TCA file.
Areas of well show poor segmentation and guide assignment	Verify the cell confluency is not too high in these areas of the well (typically near the edges or well corners). Make sure cells are well distributed after seeding to ensure even cell seeding that can minimize these issues.
Misidentification of guides to cells	• To minimize cross-talk of guides, do not use overlapping guide sequences in the same run. • Make sure to prepare samples properly and minimize cell lysis. • Use cells at ideal confluency. • Ensure cells are segmented well using the appropriate segmentation model.
Cytos Guide RNA Control Primers not showing the expected counts	Confirm the guide RNA Control Primers were added to the specific well position in the cartridge at the recommended concentration before the run and mixed well by pipetting 15 times using a P-1000 pipette.
Majority of cells are not segmented indicating cell segmentation failure	• Review the Thumbnails panel to view cell paint images. • Perform resegmentation using a custom model or an appropriate model for the specific cell type. See Resegmentation Tutorial.
Imaging displays bright, scattered fluorescent spots or a fluorescent smear	• Clean the flow cell and the nest as described in this guide. • Be sure to change your gloves before wiping the assembled flow cell to minimize any smudges onto the flow cell. • See Knowledge Base for details on minimizing background fluorescent signals.

Consumables and Tools

This section lists available Cytos kits, tools, and user-supplied consumables. Promptly store the components at the specified temperatures upon receipt. For Safety Data Sheet (SDS) information, see elementbiosciences.com/resources.

Cytos Optical Pooled Screening Kit

Part Number and Kit Name		Quantity	Shipping	Storage
860-00058	Cytos Optical Pooled Screening Kit			
	Cytos Optical Pooled Screen Cartridge (820-00046)	1	-25°C to -15°C	-25°C to -15°C
	Cytos Guide RNA Control Primers (830-00071)	1	-25°C to -15°C	-25°C to -15°C
	AVITI Buffer Bottle (820-00002)	1	Room temperature	Room temperature

Cytos Protein Kit

Part Number and Kit Name		Quantity	Shipping	Storage
860-00057	Cytos Protein Kit[1]			
	Cytos Protein Cartridge (820-00048)	1	-25°C to -15°C	-25°C to -15°C
	AVITI Buffer Bottle (820-00002)	1	Room temperature	Room temperature

[1] For assessing protein with an RNA run. See *Cytos Protein User Guide (MA-00095)*.

Cytos Protein Panels

Part Number and Panel Name		Theme	Shipping	Storage
860-00070	Cytos Protein Neuro Morphology Panel	Neuroscience	-25°C to -15°C	-25°C to -15°C
860-00071	Cytos Protein Expanded Morphology Panel	Morphology	-25°C to -15°C	-25°C to -15°C
860-00072	Cytos Protein Alternative Morphology Panel	Morphology	-25°C to -15°C	-25°C to -15°C
860-00073	Cytos Protein Immune Subtyping Panel	Immunology	-25°C to -15°C	-25°C to -15°C
860-00075	Cytos Protein Cell Signaling Panel	Cell biology	-25°C to -15°C	-25°C to -15°C

Cytos Slide Kits

Part Number and Kit Name		Quantity	Shipping	Storage
860-00064	Cytos Slide Kit, Uncoated – 1 Well (2-pack)	2	Room temperature	Room temperature
860-00065	Cytos Slide Kit, Uncoated – 12 Well (2-pack)	2	Room temperature	Room temperature
860-00066	Cytos Slide Kit, Uncoated – 48 Well (2-pack)	2	Room temperature	Room temperature

AVITI24 Flow Cell Assembly Kits

Part Number and Kit Name		Quantity	Shipping	Storage
860-00067	AVITI24 Flow Cell Assembly Kit, 1 Well or 48 Well (2-pack)	2	Room temperature	Room temperature
860-00068	AVITI24 Flow Cell Assembly Kit, 12 Well (2-pack)	2	Room temperature	Room temperature

Additional Tools

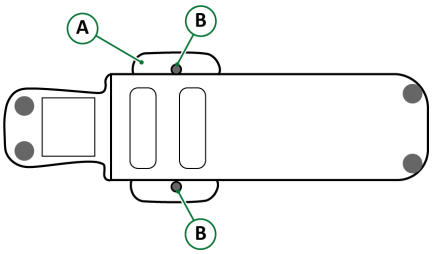
Part Number and Kit Name		Quantity
860-00033	Flow Cell Assembly Tool Set – Flow Cell Aligner (810-00016) – Flow Cell Sealer (810-00017)	 1 1
860-00069	Cytos Centrifuge Adapter (2 pack)	2

User-Supplied Consumables

Consumable	Supplier
Biological-grade/RNase-free water	General lab supplier
10 mM Tris-HCl, pH 8.0 with 0.1 mM EDTA (Low TE buffer)	General lab supplier
Isopropyl alcohol wipes	Prosat, catalog # PS-911 or similar
Lens wipes	Fisher, catalog # 11-995 or similar
Kimwipes, lint-free	General lab supplier
Compressed air duster	General supplier
Isopropyl alcohol	General supplier
Ethanol (EtOH), biological grade	General lab supplier
Phosphate-buffered saline (PBS), 1X, pH 7–7.4	General lab supplier
Pipette tips	General lab supplier
Pipettes, 8-channel or 16-channel, for 48-well slide kits Pipettes, 8-channel, for 12-well slide kits Pipette, single, for 1-well slide kits	ThermoFisher Scientific, catalog # TS 4662090 General lab supplier General lab supplier
Reagent trough	General lab supplier

Maintain the Flow Cell Sealer

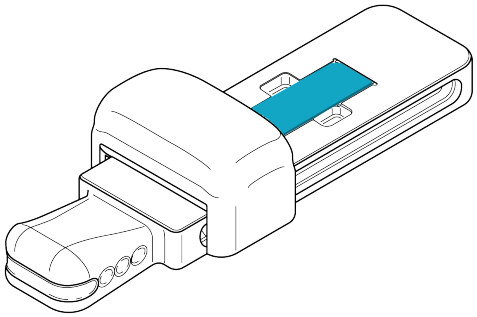
- Make sure the entire top surface of the flow cell sealer is free of dust and debris before each use.
- To inspect the surface, make sure no slides are present, and then move the roller grip forward and backward. Use compressed air to clean under the roller grip.
- If a slide has ever been damaged on the flow cell sealer:
  - » Clean the recessed slide holder with isopropyl alcohol wipe and ensure there is no debris from the slide.
  - » Disassemble and clean under the roller grip as follows:
    - ◦ From the bottom of the sealer, use a 3 mm hex head Allen key to loosen two captive screws and remove the roller grip cover. See video for details.
    - ◦ From the top of the sealer, use an isopropyl alcohol wipe to clean the roller wheel.
    - ◦ Tighten the two captive screws to reattach the roller grip cover.



A Roller grip cover

B Captive screws

- Always store the flow cell sealer with a molded placeholder slide seated in the indented surface of the sealer with the roller grip closest to the handle.



Document History

Revision	Description of Change
October 2026 Document # MA-00096 Rev. A	Initial release.

Technical Support

Visit the User Documentation page on the Element Biosciences website for additional guides and the most recent version of this guide. For technical assistance, contact Element Technical Support.

Website: www.elementbiosciences.com

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Regional Phone Numbers: www.elementbiosciences.com/contact

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