



Cytos[™] Protein

User Guide

FOR USE WITH

AVITI24[™] System
AVITI[™] Operating Software v4.1 or later
Cytos Protein Kit
Cytos Protein Panels
Cytos Custom Protein Probes

ELEMENT BIOSCIENCES

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Overview

A Cytos cell screening run configured for protein detection with the Cytos Protein Kit uses the native AVITI24 system imaging technology paired with immunofluorescence-based chemistry to capture morphology and protein targets at subcellular resolution.

The workflow requires prepared cell samples and Cytos consumables, and supports detection of up to 20 user-selected protein markers and 4 fixed cell paint markers. Protein runs typically complete in 9–24 hours depending on plexity, with onboard feature extraction via CellProfiler producing over 100 features per target. The Cytos Protein assay can run independently in a single cartridge configuration to screen protein targets for compatibility or in combination with Cytos RNA assays in a multi-cartridge configuration for multimodal profiling.

Fluorescence-based imaging chemistry on the AVITI24 System for cell paint and protein detection 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.

The Cytos Protein Kit also enables screening and optimization of up to 20 custom biotinylated primary antibodies allowing you to select the optimal custom antibody for a Cytos assay on an AVITI24 System.

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

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

This guide includes preparing reagents, assembling the flow cell, and performing a protein detection 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 multi-cartridge workflow for RNA detection with an application-specific RNA cartridge is documented in different guides. See [Experimental Planning on page 10](#).

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 protein detection can be performed **with or without** RNA 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 and 3' transcriptome sequencing that bind to RNA transcripts which are extended into cDNA, circularized, and then amplified using rolling circle amplification (RCA) to form colonies.

- **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.

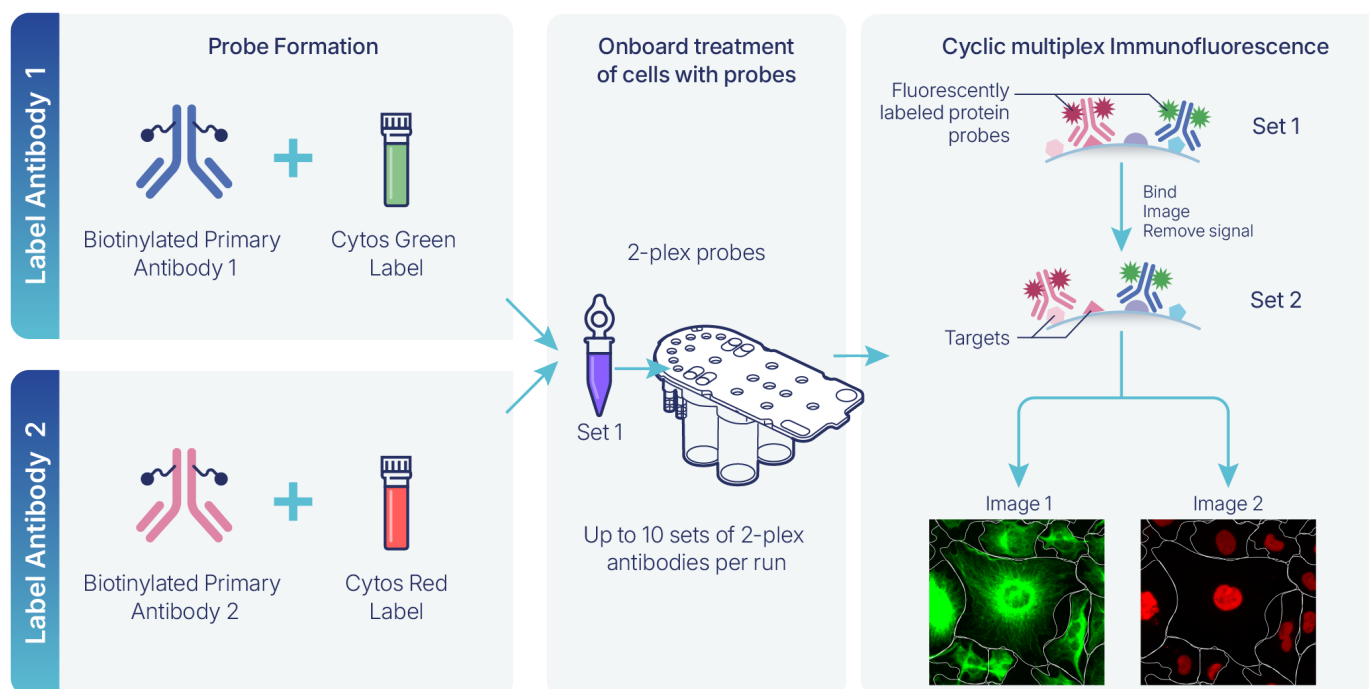
Protein Run

Cell Paint 4-Plex	Protein #Targets
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Protein Detection

The Cytos Protein Kit enables fluorescence intensity-based detection of protein targets directly from a biological sample without any library preparation steps. Cytos protein detection preserves the spatial and molecular context, and integrates with multiomic readouts. The Cytos protein workflow detects protein targets using dye-labeled fluorescent probes and includes Cytos protein panels or custom protein probes.

Each probe consists of a biotinylated primary antibody bound to a fluorescent reporter. One red dye-labeled protein probe and one green dye-labeled protein probe are pooled together for simultaneous 2-plex detection. On the AVITI24 instrument, pooled protein probes bind to their targets in the cell and are imaged. The signal is removed and a new pool of protein probes are bound and imaged in the next cycle. This cycle of probe binding, imaging, and signal removal repeats to enable detection of additional protein targets. The output of the Cytos Protein assay is a readout of fluorescent signal intensity and localization, reflecting the abundance and spatial distribution of each protein target within the sample.



Note: Colors shown in tubes are for illustration only and do not represent the actual reagent colors.

Key Terms

The key terms used in this user guide for protein reagents are defined as follows:

Probe: A single fluorescent reagent unit consisting of a biotinylated primary antibody coupled to a fluorescent reporter. Each probe targets one specific protein and carries a red or green fluorescent label.

Set: A paired combination of one red-labeled probe and one green-labeled probe, pooled together for simultaneous 2-plex detection in a single imaging cycle. Each set is loaded into a specific cartridge well in a defined order.

Panel: A predesigned, validated 4-plex grouping of protein targets (two Sets), grouped by fluorescent color compatibility and built for a specific application. Panels are ordered as a kit and used alone or combined with custom probes (up to 20 total targets per run).

Cell Screening Run Consumables

A protein run on the AVITI24 System requires a Cytos Protein Cartridge and buffer bottle, a slide kit, and flow cell assembly kit. To perform a dual flow cell run, a quantity of two of each kit is required. In addition, Cytos protein panels or custom protein probes are also required.

For a list of kits with catalog numbers, see [Consumables and Tools on page 38](#).

Cytos Protein Cartridge

The Cytos Protein Cartridge provides reagents for a Cytos protein run including cell paint reagents and controls with the exception of protein targets. The cartridge allows addition of up to 20 protein targets to support your experimental goals. The positive control, TOMM20, is a mitochondrial probe included in the cell paint reagents which shows strong expression in all cells. The negative control probe set includes probes targeting Mouse IgG and Rabbit IgG for generating a baseline for non-specific expression.

For protein target options, select from predesigned and validated Cytos protein panels or design your own custom protein panel with up to 20 custom protein targets. To prepare custom protein targets, see [Prepare Custom Protein Probes on page 13](#).

Cytos Protein Panels

The Cytos protein panels are 4-plex protein panels designed for use with a Cytos Protein Cartridge for a Cytos protein detection run. Each set in the protein panel is grouped based on fluorescent probe color compatibility. Five Cytos protein panels are available that cover a broad set of applications such as immunology, morphology (includes expanded, alternative and neuro), and cell signaling. See [Cytos Protein Panels on page 38](#) for available panels for use with the Cytos Protein cartridge.

For a list of targets associated with each 4-plex protein panel, see [Cytos Protein Panel Targets \(MA-00099\)](#).

Cytos Custom Protein Probes

Cytos Custom Protein Probes allow you to screen and optimize your biotinylated primary antibodies for use in a Cytos protein run. Cytos Custom Protein Probes include reagents for labeling your biotinylated primary antibody with a fluorescent reporter (Cytos Red Label and Cytos Green Label) and blocking (Cytos Biotin Reagent). A red-labeled and a green-labeled custom probe is then pooled to form a 2-plex custom protein probe set. The custom protein probe set is added to the Cytos Protein Cartridge for protein detection. See [Prepare Custom Protein Probes on page 13](#) and [Add Protein Panels to the Cartridge on page 20](#).

Protein Selection Options

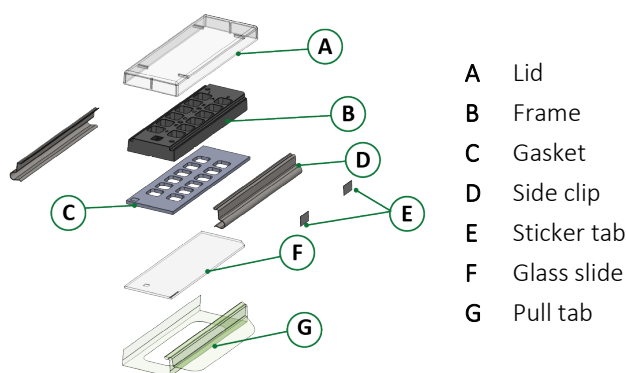
Cytos protein panels along with custom protein probes provide configurable options to meet variable experimental needs.

The following table lists available Cytos protein panel options. In addition to cell paint, the morphology protein panels offer additional options to capture cell features with different stains. See [Cytos Protein Panels on page 38](#).

Protein Panel	Recommended Usage
Cytos Protein Expanded Morphology Panel	Use for deeper morphological profiling as this panel adds new organelle markers not included in the core morphology panel (cell paint)
Cytos Protein Alternative Morphology Panel	Use with some primary or non-immortalized cell lines to allow for resegmentation if the core morphology panel does not provide expected segmentation and staining
Cytos Protein Neuro Morphology Panel	Use with neural cell types such as iPSC-derived neurons, microglia, and astrocytes to support improved segmentation and staining
Cytos Protein Immune Subtyping Panel	Use with samples with mixed immune cell populations to distinguish immune cell subsets via lineage and activation markers
Cytos Protein Cell Signaling Panel	Use to assess MAPK pathway activation and stress response signaling

Cytos Slide Kit

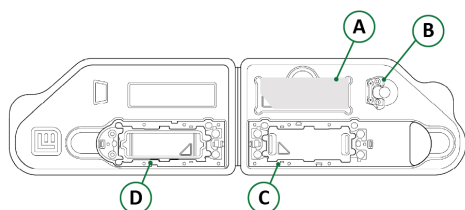
The Cytos Slide Kit is used for culturing cell samples onto the slide. The slide kit includes a glass slide with a barcode for tracking and validation, a frame, a gasket, two side clips, pull tab, and a lid. The frame and the gasket determine the number of wells on the slide. Before starting a Cytos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



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 23](#).

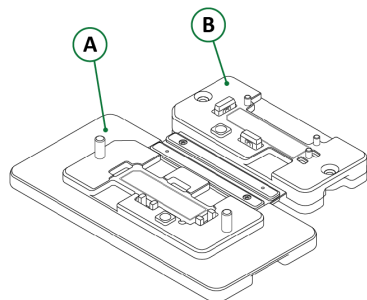


- A Adhesive slide
- B Flow cell gaskets (2)
- C Frame bottom
- D Frame top

Flow Cell Assembly Tool Set

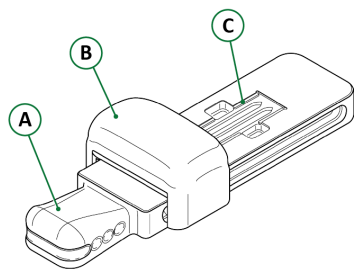
The Flow Cell Assembly Tool Set includes tools to convert the Cytos Slide Kit into a flow cell using parts provided in the AVITI24 Flow Cell Assembly Kit.

- **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 24](#).



- 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 40](#).

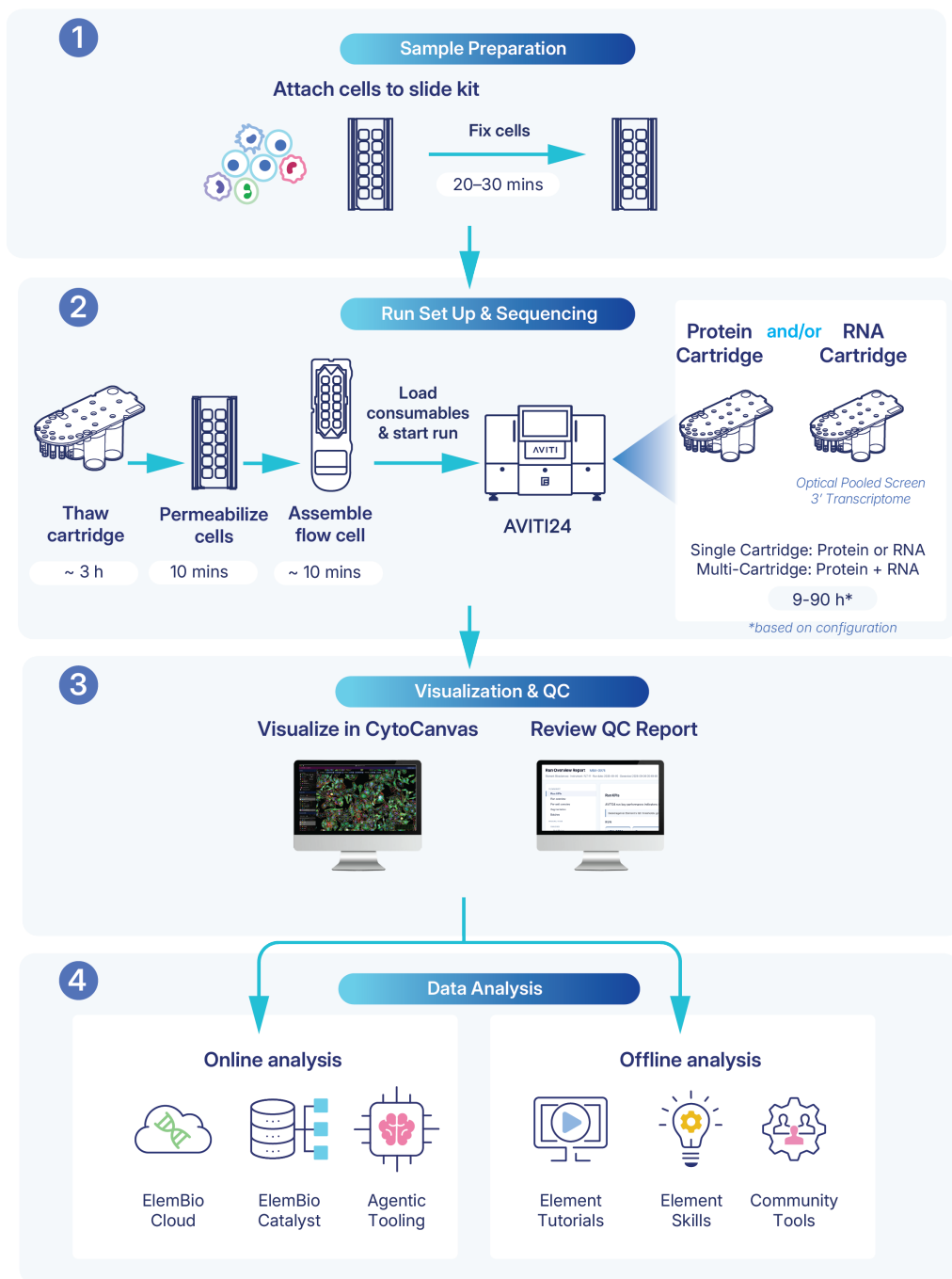


- A Base grip
- B Roller grip
- C Indentation for slides

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 a protein detection run using the Cytos Protein 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, RNA 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 protein detection without RNA detection, use the protein cartridge workflow in this guide.
- **Multi-Cartridge Workflow:** For RNA detection following protein detection, **first** complete the protein cartridge workflow in this guide and then refer to the Cytos RNA assay user guides listed in the following table to complete the RNA multi-cartridge workflow.

The following materials are required for performing a protein run. For details and storage conditions, see [Consumables and Tools on page 38](#). For details and supplier information on materials supplied by the user, see [User-Supplied Consumables on page 39](#).

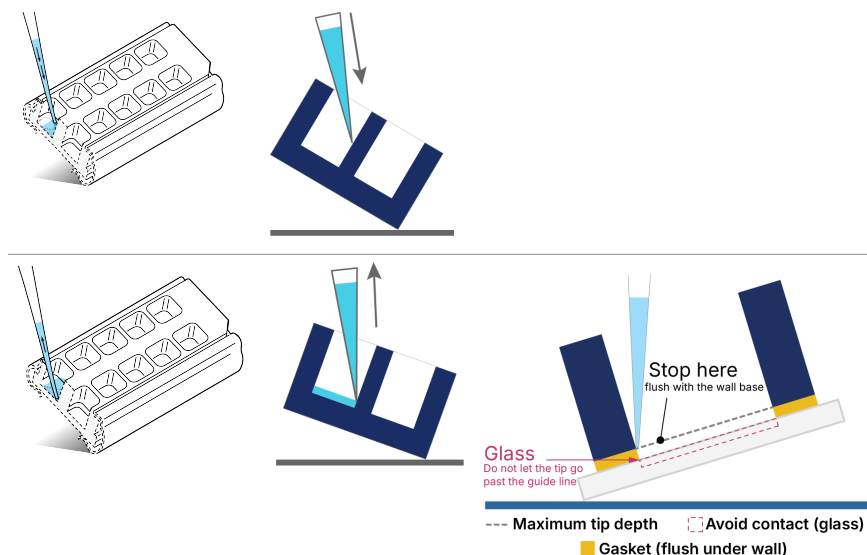
Materials	Included with a Kit / User Supplied
Cytos Slide Kit with fixed cells	See Cytos Sample Preparation & Optimization User Guide (MA-00094)
For Cytos RNA assay	See Cytos Optical Pooled Screening User Guide (MA-00096) , Cytos 3' Transcriptome User Guide (MA-00098) , or Cytos Multimodal Pooled Screening User Guide (MA-00097)
For protein assay	
Cytos Protein Kit	Included with the kit
Cytos Protein Panels (if using protein panels in assay)	Available for purchase
Cytos Custom Protein Probes (if using custom probes in assay)	Available for purchase
Biotinylated custom primary antibody (if using custom probes in assay)	User Supplied
RiboLock RNase Inhibitor (if your assay includes RNA detection)	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	User Supplied
Isopropyl alcohol (IPA) wipes, lens wipes, and lint-free Kimwipes	User Supplied

Sample Preparation

The Cytos Protein detection 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 the Protein Workflow

A Cytos protein run requires Cytos protein panels and/or custom protein probes. Each set in the Cytos protein panel is grouped based on fluorescent probe color compatibility. Use the [ElemBio Cloud Custom Designer™](#) to design your run with up to 20 protein targets using a combination of Cytos Protein panels and/or custom protein probes.

The Cytos Protein run can be designed **with or without** RNA detection.

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 **Protein**.

» In the Protein module, choose up to a maximum of 20 protein targets. Select from a choice of available Cytos protein panels or add custom protein probe names.

» [Optional] Select **RNA** if you plan to include RNA detection and then select Guide RNA or 3' Transcriptome.

- In the Guide RNA Sequencing module (if using Guide RNA), define sequencing target sites to generate compatible oligo sequences for the RT primer and sequencing primer. Create a Target Cell Assignment (TCA) manifest.
- Order the RT primer and sequencing primer with the specified sequences from a third-party oligo vendor for CRISPR analysis.

4. Order Cytos Protein Panels. See [Cytos Protein Panels on page 38](#).

To prepare custom protein probes, see [Prepare Custom Protein Probes on page 13](#).

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.

Prepare Custom Protein Probes

If you are not using custom proteins in your assay, proceed to [Run Setup for Protein Cartridge Workflow on page 18](#) or [Multi-Cartridge Workflow on page 34](#).

Preparing custom protein probes involves diluting the biotinylated custom primary antibodies at a required concentration. Combine diluted custom primary antibodies with Cytos probe labels and Cytos Biotin Reagent to form custom primary antibody probes. Mix a red custom primary antibody probe with a green custom antibody probe to form 2-plex custom antibody probe set which is used in the protein run.



IMPORTANT

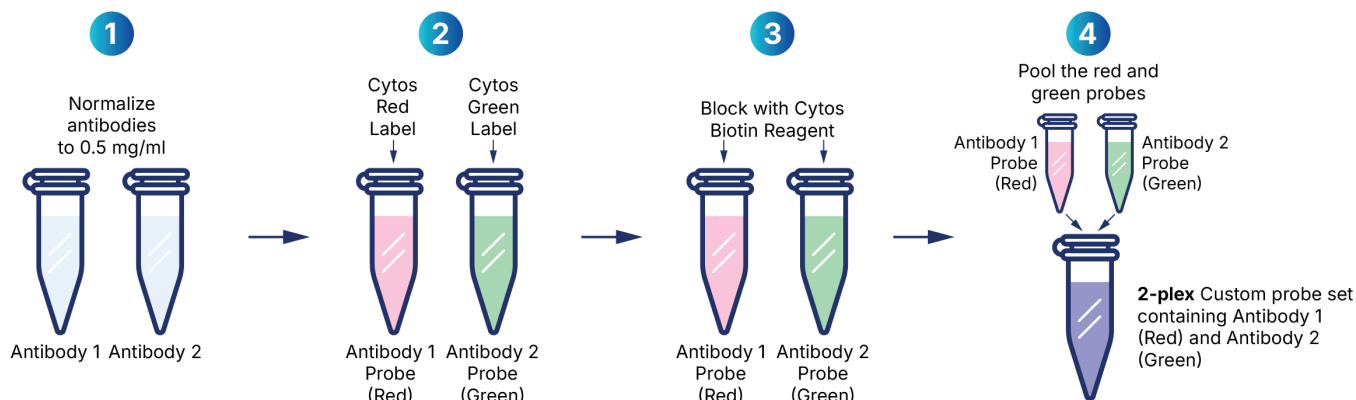
Prepare custom protein probes fresh on the day of use for best performance.

Prerequisites

1. Confirm that primary antibodies are **biotinylated** and are compatible.
 - » For optimal performance, use monoclonal antibodies with site-specific biotinylation, visit [Element Biosciences](#) for a list of biotinylated antibodies that are tested and available for the Cytos platform.
 - » Select custom primary antibodies that are validated by immunocytochemistry/immunofluorescence or flow cytometry to increase the compatibility with Cytos assay.
2. No reducing agents in the antibody storage buffer.
3. Determine plexity based on the total number of custom primary antibodies that you plan to screen with this protocol.

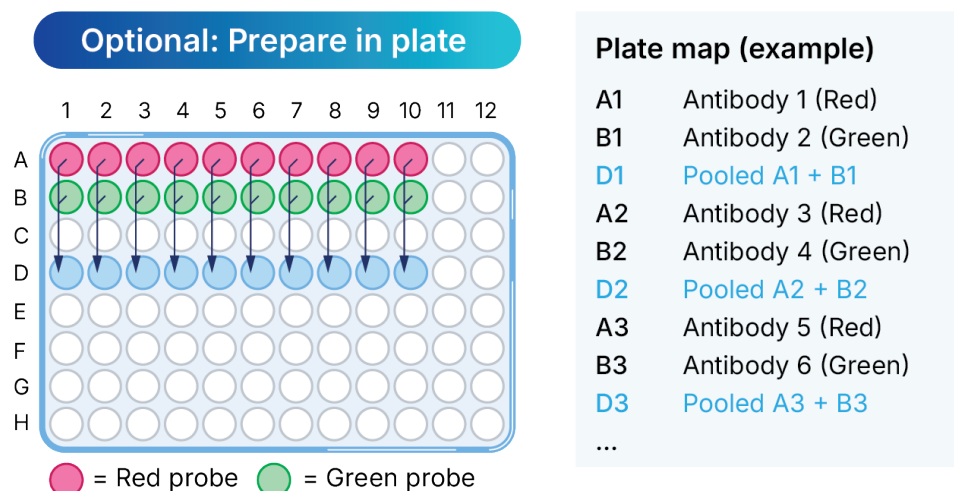
Protocol Summary

The following diagrams illustrate custom probe preparation using tubes or plates. When working with multiple custom proteins, **maintain clear labeling and organization** throughout the workflow to prevent mix-ups.



Note: Colors shown in the tubes and plate are for illustration only and do not represent the actual reagent colors.

If using plates for custom probe preparation, prepare red and green probes for each custom antibody in separate wells and then pool each red and green probe pair into a designated well in row D, as illustrated in the following diagram.



Protein Handling Guidelines

- **Always store and maintain protein reagents at 4°C or on ice while working.**
- Do not mix by vortexing. Always use a pipette to mix contents in the tube and gently mix by pipetting 10 times.
- Protect the reagents from light.
- Store the Cytos Protein Panels at -25°C to -15°C in a manual defrost freezer and avoid freeze-thaw cycles.
- Use RNase-free pipette tips.

Thaw Reagents and Dilute Primary Antibodies

1. Remove the following kit components from -25°C to -15°C storage:
 - » Cytos Red Label
 - » Cytos Green Label
 - » Cytos Biotin Reagent
 2. Thaw the reagents at room temperature. Once thawed, store on ice until use.
 3. Thaw each biotinylated custom primary antibody on ice, if stored frozen.
 4. Briefly centrifuge the antibody tube and pipette to mix.
 5. Perform serial dilutions of your biotinylated custom primary antibody in 1X PBS as needed to achieve a stock concentration of 0.5 mg/ml. Store on ice.
- If you used plates for primary antibody dilutions, briefly centrifuge the plate for 1–2 minutes to remove any bubbles.

Label Custom Proteins with Cytos Probes



IMPORTANT

Prepare custom protein probes fresh on the day of use for best performance.

This protocol prepares ~ 1.2 ml custom probe solution containing 4 nM of each custom probe for one cell screening run on an AVITI24. Scale all reagent volumes proportionally for additional runs or higher final custom probe concentrations.

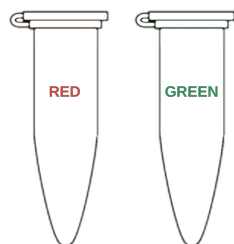
The molarity in this section is calculated assuming an IgG molecular weight of 150 kDa.



NOTE

If you are performing a protein optimization experiment, you can prepare your primary antibody in a final concentration range of 2–40 nM.

1. Gather the following consumables:
 - » 1X PBS
 - » PCR tubes, PCR strip tubes, 96-well plates, or microcentrifuge tubes (use low-bind tubes for reagent preparations)
2. For each 2-plex custom antibody set, label one PCR tube as Red and one PCR tube as Green with the target protein names. See [Protocol Summary on page 13](#) for an example plate map for labeling guidance, if using a plate to prepare custom probes.



If you are preparing 1-plex custom primary antibody probe, label only one tube and adjust the protocol for preparing 1-plex probe.

3. Add **3.6 µl** 1X PBS to each tube.
4. To the tube marked as red, add **4 µl** Cytos Red Label.
5. To the tube marked as green, add **4 µl** Cytos Green Label.
6. Pipette 10 times to mix. Do not vortex. Briefly centrifuge the tube to collect the contents.
7. To the custom antibody tube labeled as red, add **2.4 µl** of 0.5 mg/ml of biotinylated custom primary antibody.
To the custom antibody tube labeled as green, add **2.4 µl** of 0.5 mg/ml of biotinylated custom primary antibody.
8. Pipette 10 times to mix. Do not vortex. Briefly centrifuge the tube to collect the contents.
9. Incubate at room temperature for **1 hour**.
10. Add **10 µl** Cytos Biotin Reagent to each tube to block any unoccupied binding sites.
11. Pipette 10 times to mix. Do not vortex. Briefly centrifuge the tube to collect the contents.
12. Incubate at room temperature for **30 minutes**.

Each 1-plex custom protein probe is at 400 nM concentration in a total volume of 20 µl.

Proceed to [Pool Custom Protein Probes on page 16](#) to prepare 2-plex custom protein probes at 4 nM concentration.

Pool Custom Protein Probes

Pool the red and green label probes to prepare a 2-plex custom protein probe set at final probe concentration of 4 nM.

 **IMPORTANT**
Pool only one red and one green labeled probes per set using your published design package from ElemBio Cloud as a guide. Do not pool probes with the same color label.

Maintain clear labeling and organization throughout the workflow to prevent mix-ups.
For a 1-plex custom protein probe, use 1X PBS instead of a second probe.

1. Gather the following consumables:
 - » 1X PBS
 - » 40 U/μl RiboLock RNase inhibitor (if using RNA detection)
2. Label a tube for the 2-plex protein probe clearly with protein name to minimize any tube swapping especially if you are labeling more than one custom primary antibody.

 **NOTE**
If your assay includes RNA detection, use RiboLock RNase inhibitor instead of 1X PBS.

3. Add the appropriate volume of reagents in the following order for a total volume of **1188 μl**.

Reagent	RNA Detection	No RNA Detection
1X PBS	1158 μl	1164 μl
Red custom protein probe	12 μl	12 μl
Green custom protein probe	12 μl	12 μl
RiboLock RNase inhibitor	6 μl	--

4. Pipette 10 times to mix. Do not vortex. Briefly centrifuge the tube to collect the contents.
5. Store on ice until use. Use the custom protein probe fresh on the same day for best performance.
6. Proceed to [Run Preparation on page 17](#).

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 ordered protein panels or **freshly prepared** custom protein probes.

Protocol Summary

Run Preparation

- 1 Thaw reagent cartridge
- 2 Thaw applicable protein panel

Thaw Reagent Cartridge

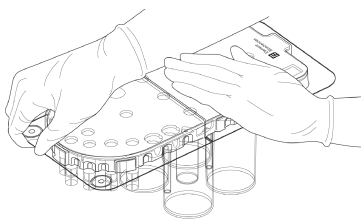
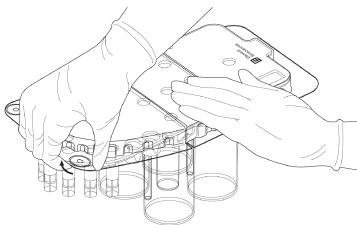
1. Remove a cartridge from -25°C to -15°C storage.



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 Protein Panel Tubes

Skip this step if your assay includes only custom protein probes.

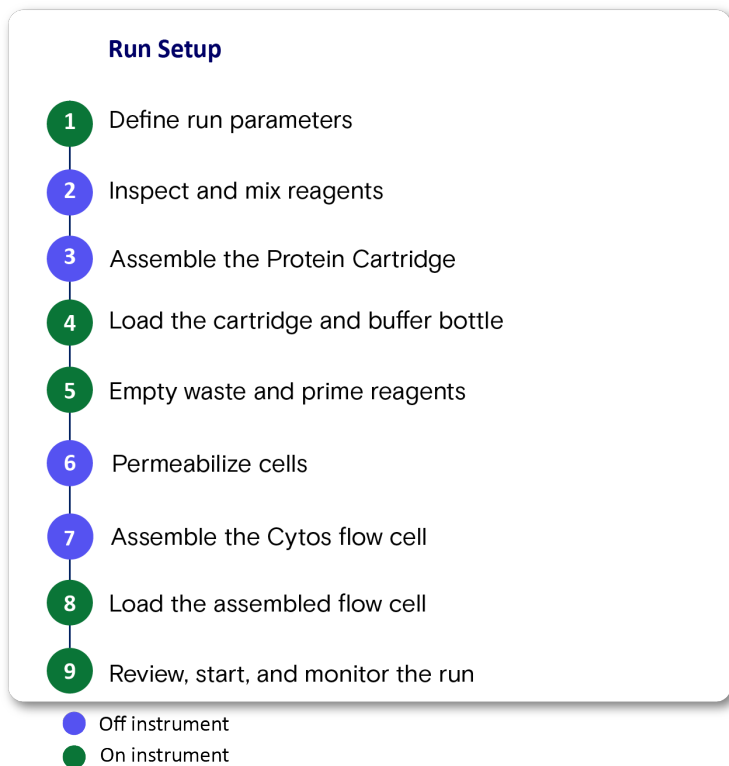
1. When the reagent cartridge is almost thawed, remove the applicable Cytos Protein Panels from -25°C to -15°C storage.
2. Thaw reagents in a room temperature water bath for 15 minutes. Store on ice until use.

Run Setup for Protein Cartridge Workflow

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.
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 **Protein Screen**.
 - 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.
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.

Add Protein Panels to the Cartridge

The Cytos Protein Panels and custom protein probes are added to the Cytos Protein Cartridge in a **specific order and position** to ensure proper processing during the run.

A printable cartridge guide that shows the order of loading reagents onto the cartridge is available as part of your published design package from ElemBio Cloud.

IMPORTANT
Cytos Protein Panels and custom protein probes are always added only in the following wells in the specific listed order.

Well Loading Order	Cartridge Map	Loading Guidance
1		Always begin adding proteins starting from position 1 of the cartridge.
2		Load proteins only in wells in the order as shown in the map and do not add proteins in any other cartridge wells.
3		Ensure you load protein in well 8 before well 5.
4		Proteins are loaded as a 2-plex set (paired set with Red and Green label per well). For example: Set 1 is the first set from a Cytos protein panel loaded in well 1 followed by Set 2 which is the second set from the same Cytos protein panel.
8		Load Set 1 in wells shown in light gray followed by Set 2 in wells shown in dark gray.
5		
6		
7		
C		
D		

CAUTION
Incorrect order of protein loading on the cartridge can result in incorrect biological conclusions and unreliable data.

1. Briefly centrifuge the protein panel tubes to collect the contents and pipette to mix.
2. Wipe any excess water on the cartridge lid or exposed foil to avoid contamination during the reagent loading process.
3. Using a clean pipette tip, pierce the foil seal of the protein tubes in the applicable positions of the cartridge. Use a clean pipette tip each time.
4. Based on your experimental design and using the cartridge guide from ElemBio Cloud as a reference, add **1140 µl** of protein in the well in the **correct order**. Use a P-1000 pipette to add 2 x 570 µl into each well for a total of 1140 µl.

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 a protein cartridge, make sure the Cytos protein panels and/or custom protein probes 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.

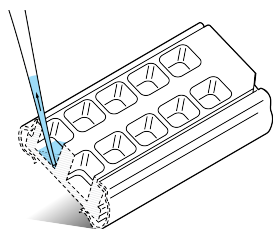
8. Select **Next** to *automatically* start priming. Priming takes approximately 27 minutes.
9. While priming is progressing, proceed to [Permeabilize Cells on page 22](#).

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.

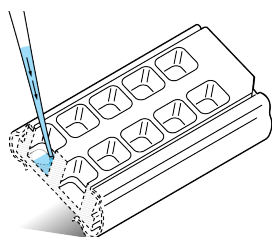
NOTE See [Sample Preparation on page 11](#) for pipetting guidelines. See [Knowledge Base](#) for detailed videos on pipetting guidance.

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.

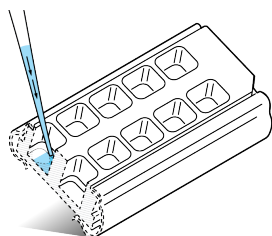
Reagent	48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
1X PBS	50 μ l	200 μ l	2 ml



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.

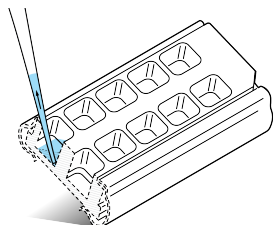
Reagent	48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
Permeabilization reagent	50 μ l	150 μ l	1.5 ml



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 μ l	200 μ l	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 μ l tip (recommended)
- Flow cell aligner and flow cell sealer
- AVITI24 Flow Cell Assembly Kit

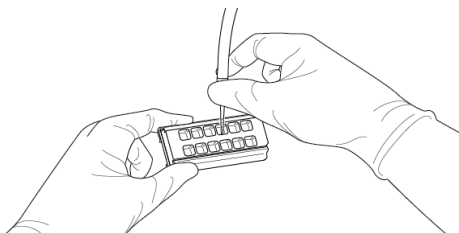


IMPORTANT

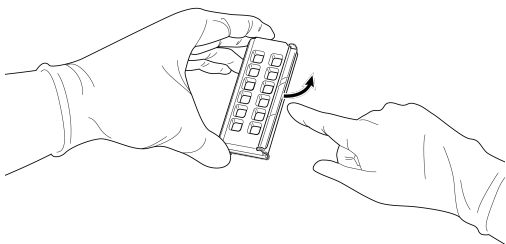
For best results, load the flow cell within 10 minutes of assembly. Do not allow the flow cell to dry.

Disassemble the Slide Kit

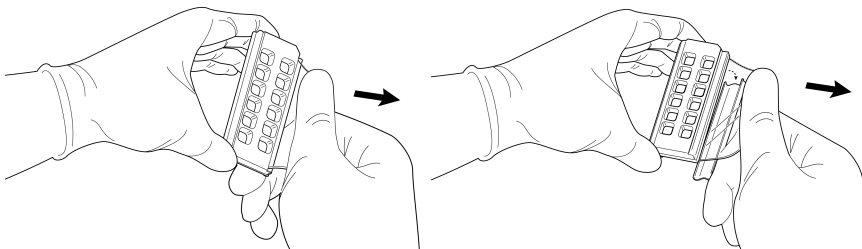
1. Wear a clean pair of gloves before disassembling the slide kit.
2. Use a vacuum aspiration system with a 200 μ l tip to remove the liquid from each well of the slide kit:
 - a. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
 -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



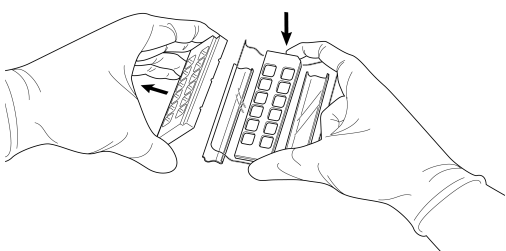
3. Hold the slide kit over the bench so clips do not fall to the ground during disassembly. Hold one side of the slide kit along the metal clip. Take care to avoid placing any pressure on the slide.
See the [video](#) on slide kit disassembly.
4. To remove the metal side clips, locate the plastic pull tab on the side of the slide kit and grip the pull tab to remove it from the adhesive.



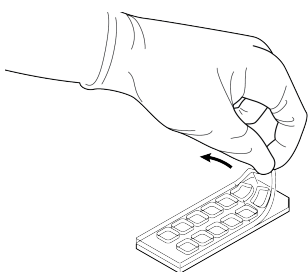
5. Grab the pull tab flap and pull the flap outwards with a firm force to dislodge the metal clip closer to the pull tab. Apply even force and avoid pulling from one spot of the pull tab.



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

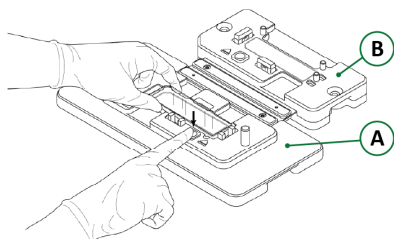
1. Make sure the surface of the flow cell aligner is clean. Thoroughly wipe both sides of the aligner with ethanol, including all pins.



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

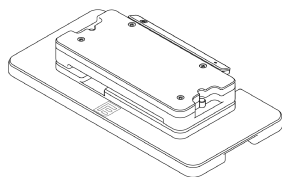
4. Open the AVITI24 Flow Cell Assembly Kit and remove the adhesive slide from the package.



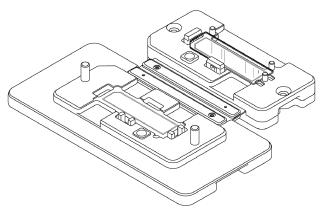
CAUTION

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.

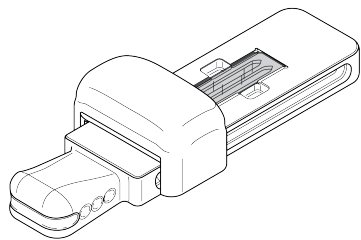


10. Clean the surface of the flow cell sealer with an isopropyl alcohol wipe. Thoroughly wipe the recessed slide holder.
For more information, see [Maintain the Flow Cell Sealer on page 40](#).

**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.

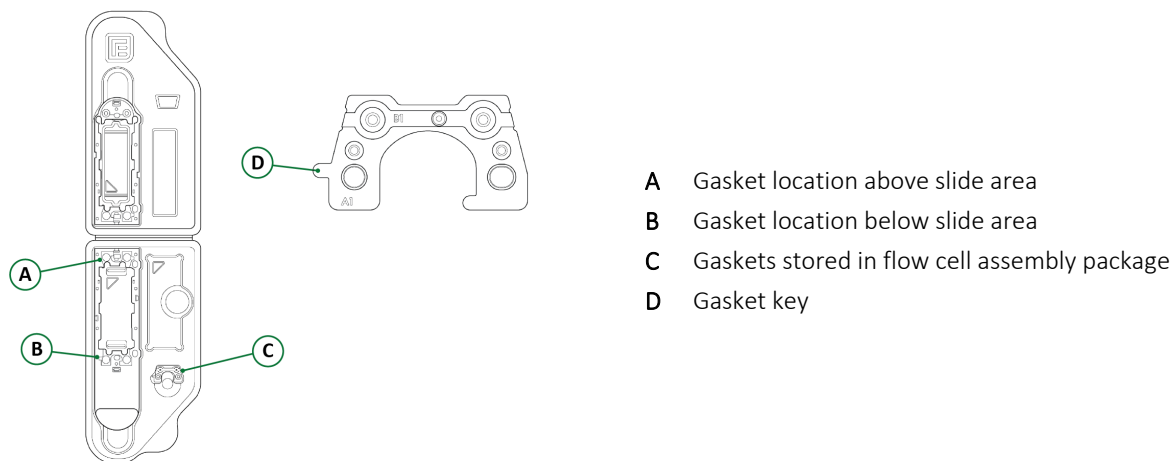
**NOTE**

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.

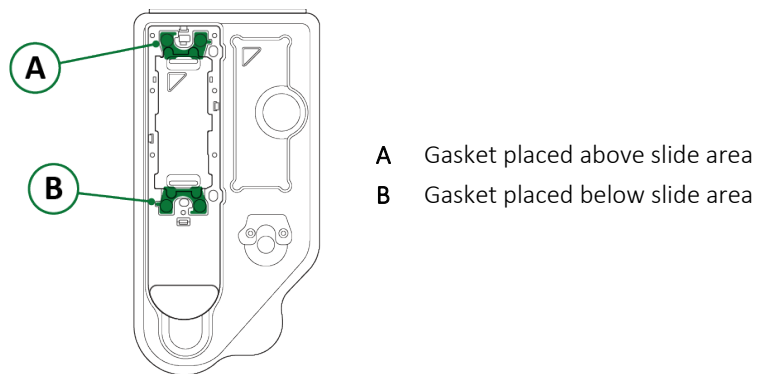
Assemble the Flow Cell Cassette

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

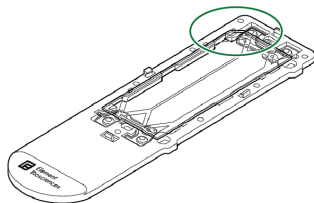


2. Position each of the two flow cell gaskets onto the bottom frame of the flow cell cassette, one above and one below the slide area. Make sure the gasket key is properly seated in the recess.

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

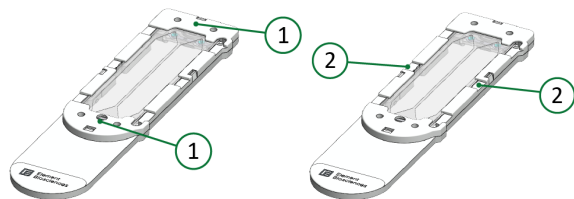


3. Remove the slides from the flow cell sealer.
4. Place the sealed slides onto the bottom frame of the flow cell cassette with the beveled corner in the top-left position as shown on the packaging. The cassette design ensures only one orientation. Make sure the slides rest flat on the bottom frame.



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](#).

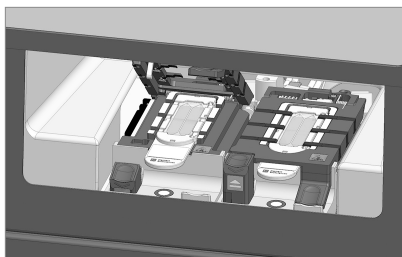
Load the Flow Cell



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 32](#).
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. [Optional] If you imported run manifests from a USB drive, disconnect the USB drive.
5. 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 31](#).
 - » If you removed a wash tray, follow the guidelines for wash tray maintenance in the user guide for your instrument.

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.

Review Protein Run

AVITI24 performs onboard analysis providing analysis-ready outputs after a Cytos Protein run. For a **single protein cartridge** run, proceed to [Data Analysis on page 35](#).

If you are performing a **multi-cartridge** run using a second RNA cartridge, review run results to quickly assess the protein run performance. At the end of the protein batch, run metrics and thumbnails appear onscreen on the instrument or on ElemBio Cloud. The fluorescent intensity charts for the target, positive control, and negative controls are displayed along with cell count, cell confluency, and Assigned (%) metrics.

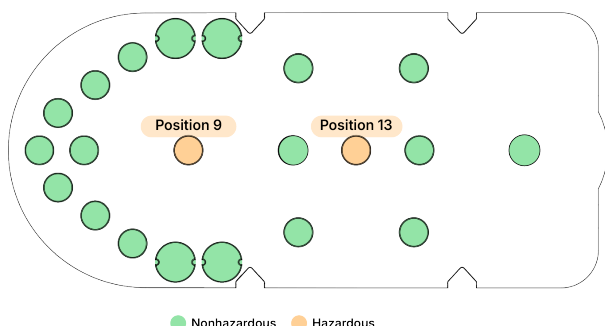
- Review the fluorescent intensity values for the controls to check that the values are in the acceptable dynamic range.
- Ensure the negative control intensity values are below the positive control.
- Ensure the target protein's intensity (measure from the Mean Intensity Above Threshold value) is above the negative control value (expected to be more than 2x greater than control).
- Review the thumbnails for cell paint, cell count, and confluency.

Proceed to [Multi-Cartridge Workflow on page 34](#) if the protein run results meet the quality control specifications and your experimental design includes RNA detection.

Discard the Cartridge and Bottle

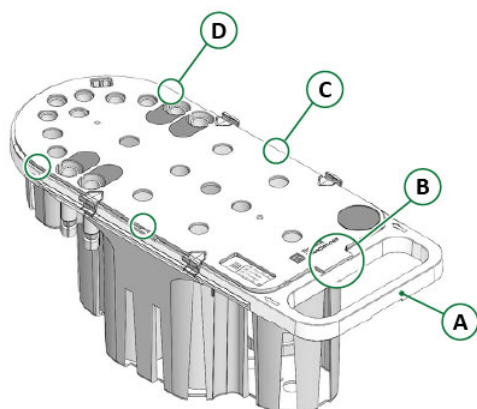
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources.

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

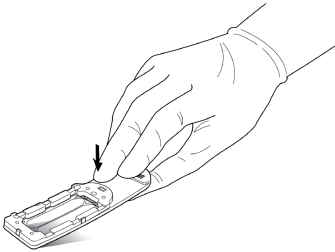
If the Flow Cell Integrity Test fails during run setup, perform the following steps to recover the flow cell.



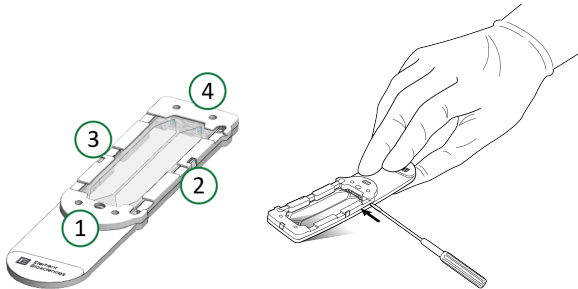
IMPORTANT

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.

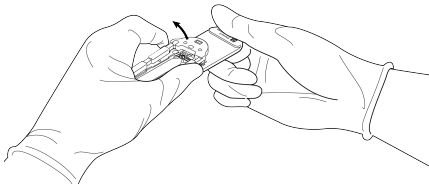
1. Open the nest and remove the flow cell.
2. Use a vacuum aspiration system or pipette at the end ports to remove all liquid from the flow cell. Make sure the flow cell is completely dry.
3. Release each of the four snap positions that secure the top half of the cassette to the bottom half in the order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

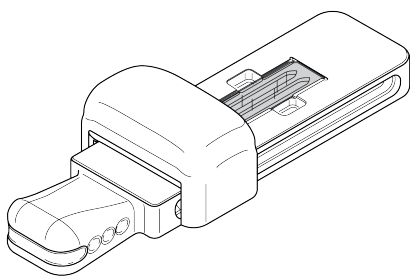


- c. With position 1 released, insert the tool at positions 2 and 3 along the side of the cassette. Gently rotate the tool to release each snap.
 - d. To release the snap at position 4, rotate the top half of the cassette upward or use the tool from the side of the cassette.



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.

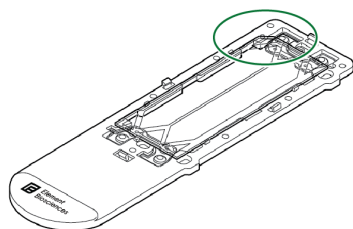
- b. Place the affixed slides in the recessed slide holder. The slides must be well-seated to avoid damage to the slides.



- 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.

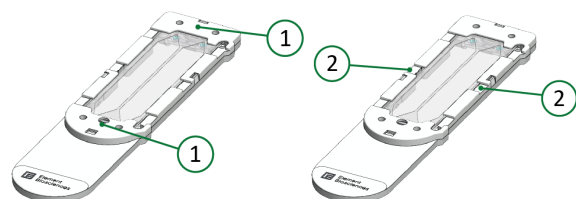
7. Place the sealed slides onto the bottom half of the flow cell cassette with the beveled corner in the top-left position. Make sure the slides rest flat on the cassette bottom.



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.

Multi-Cartridge Workflow

A multi-cartridge workflow is performed after the first Cytos Protein Cartridge workflow is complete and a quick review of the protein data 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)*.

Depending on the RNA cartridge that you plan to use for the second cartridge run, use the following application specific user guide to ensure you are using the correct protocol.

Application	Cytos Cartridge	User Guide
Optical Pooled Screening (OPS)/guide RNA detection	Optical Pooled Screen	Cytos Optical Pooled Screening User Guide (MA-00096)
Whole transcriptome screening, drug screening	3' Transcriptome	Cytos 3' Transcriptome Guide (MA-00098)
Multimodal Pooled Screening and CRISPR	Multimodal Pooled Screening	Cytos Multimodal Pooled Screening Guide, (MA-00097)

Proceed to the **Run Preparation** section in the applicable guide to start the multi-cartridge workflow which includes steps to thaw the cartridge, load the cartridge and AVITI Buffer Bottle, and then follow prompts on the AVITI OS interface to continue the run.

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 a protein run using the Cytos Protein Kit.

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

Problem	Resolution
Low signal intensity from the positive control	• Check if you are using a TOMM20 knockout or a mitochondria disrupting drug treatment that can result in underexpression of the protein target (positive control). Perform an optimization run with the Cytos Protein Cartridge using the same cell treatment conditions. • Using expired reagents, panels, or cartridge can produce lower signal intensity. Use reagents within the expiration date.
High signal intensity from negative control	• Cell debris from cell culture can contribute to false signal detection. Optimize cell culture. See Cytos Sample Preparation & Optimization User Guide (MA-00094). • Non-specific binding increases background signal. Perform an optimization run with the Cytos Protein Cartridge. • Check if your cell type contains proteins that bind to rabbit or mouse isotypes.
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.
Low signal intensity for a given target	• Target is underexpressed in the cell type due to a treatment. • Perform an optimization with the Cytos Protein Cartridge using antibody titration to find the optimal antibody concentration. • Using expired reagents, panels, or cartridge can produce lower signal intensity. Use reagents within the expiration date. • Target has degraded during the sample preparation. Follow the recommendations in this guide for handling protein and use freshly prepared custom protein probes for the assay.
Incorrect target localization	• Use the printable cartridge guide included in the ElemBio Cloud design package to load proteins into the cartridge in the correct order. • Non-specific signal masks true localization. Perform an optimization run with the Cytos Protein Cartridge.
Whole cell is unexpectedly illuminated without any targeted signal localization	Missed adding custom primary antibody or Cytos Biotin Reagent (blocking reagent). Follow the probe preparation protocol in this guide.
High background signal or diffuse background signal	• Perform cell culture optimization using the Cytos Optimization cartridge and protein optimization with Cytos Protein Cartridge to minimize non-specific signal from cell debris, proteins leaking from lysed cells, or cell loss due to improper fixation. • Minimize any high autofluorescent or aggregated protein components included in cell culture media.

Problem	Resolution
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.
High wheat germ agglutinin (cell membrane) background	Wheat germ agglutinin can bind to laminin or matrigel surface coatings or any surface that has N-acetyl-D-glucosamine (GlcNAc) or sialic acid (N-acetylneuraminic acid) causing high background but may not impact segmentation. To obtain images with less background, use Cytos Protein Alternative Morphology Panel with a Cytos Protein Cartridge, and perform resegmentation and analysis with the Alternative Morphology Panel after the run.
One of the channels is completely off or bleed through from an alternate channel indicating signal contamination between channels	• Use the printable cartridge guide included in the ElemBio Cloud design package to load proteins into the cartridge in the correct order. • Do not mix custom protein probes with Cytos protein panels in the same well of the cartridge and do not mix set 1 with set 2 of Cytos protein panels. • For custom protein probes, pool only one red and one green labeled probes. Do not pool probes with the same color label. Be sure to label the protein names on the tubes when performing the pooling step to prepare 2-plex probes. • Highly overexpressed target in one channel. Perform an optimization run with the Cytos Protein Cartridge focusing on reducing the concentration of the custom target.

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 Protein Kit

Part Number and Kit Name		Quantity	Shipping	Storage
860-00057	Cytos Protein Kit			
	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

Cytos Protein Panels

See [Cytos Protein Panels on page 6](#).

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 Custom Protein Probes

Part Number and Kit Name		Quantity	Shipping	Storage
830-00074	Cytos Custom Protein Probes			
	Cytos Red Label	1	-25°C to -15°C	-25°C to -15°C
	Cytos Green Label	1	-25°C to -15°C	-25°C to -15°C
	Cytos Biotin Reagent	1	-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

Cytos Protein User Guide

Additional Tools

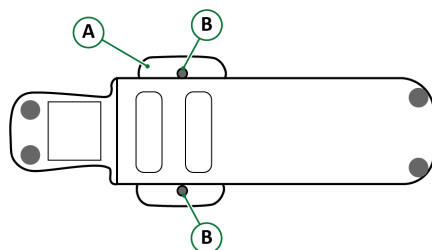
Part Number and Kit Name		Quantity
860-00033	Flow Cell Assembly Tool Set	
	– Flow Cell Aligner (810-00016)	1
	– Flow Cell Sealer (810-00017)	1
860-00069	Cytos Centrifuge Adapter (2 pack)	2

User-Supplied Consumables

Consumable	Supplier
Biological-grade/RNase-free water	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
RiboLock RNase Inhibitor	ThermoFisher Scientific, catalog # EO0381
Pipette tips	General lab supplier
Pipettes, 8-channel or 16-channel, for 48-well slide kits	ThermoFisher Scientific, catalog # TS 4662090
Pipettes, 8-channel, for 12-well slide kits	General lab supplier
Pipette, single, for 1-well slide kits	General lab supplier
Reagent trough	General lab supplier
PCR tubes, PCR strip tubes/plates, or microcentrifuge tubes	General lab supplier
Pipette tips, RNase-free	General lab supplier

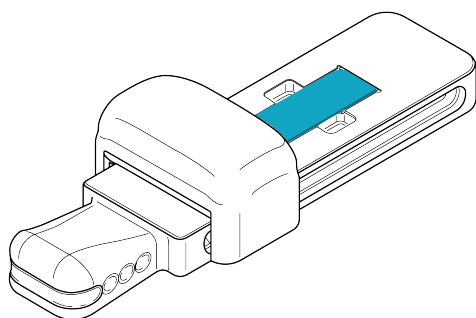
Maintain the Flow Cell Sealer

- 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-00095 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

Email: support@elembio.com

Regional Phone Numbers: www.elementbiosciences.com/contact



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