



Cytos™ Sample Preparation & Optimization

User Guide

FOR USE WITH

AVITI24™ System

AVITI™ Operating Software v4.1 or later

Cytos Onboard Optimization Kit

ELEMENT BIOSCIENCES

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Overview

This guide provides instructions for preparing samples, assembling the flow cell, and performing a cell culture optimization run with Cytos Onboard Optimization Kit. 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 a cell screening run with a specific cartridge is documented in different guides. See [Supported Protocols on page 10](#).

Cytos Onboard Optimization Kit

The Cytos Onboard Optimization Kit allows cell culture optimization after preparing cell samples on a Cytos Slide Kit. The sample assessment is performed on an AVITI24 System.

The Cytos Onboard Optimization Kit utilizes fluorescence-based imaging chemistry for cell paint detection. Cell paint uses a combination of fluorophore-linked antibodies and fluorescent dyes to label cell membrane (wheat germ agglutinin), nuclear (RedDot2), mitochondria (TOMM20), and actin (phalloidin) morphology markers. These morphology markers support cell segmentation and the extraction of quantitative cellular morphology features.

The cell culture optimization run on an AVITI24 System provides detailed cell morphology output such as cell confluency, cell area, cell diameter along with CellProfiler values. The Cytos Onboard Optimization Kit can also be used to assess compatibility of the cell line of interest with the Cytos Slide Kit before using these cell samples for a Cytos assay.

With the Cytos Onboard Optimization Kit, you can test up to 48 different cell lines or cell culture conditions in a single cell culture optimization run to evaluate compatibility of these experimental conditions with the Cytos assay.

The Cytos Onboard Optimization Kit includes the following reagents that allow for one cell culture optimization run:

- Cytos Optimization Cartridge
- AVITI Buffer

Optimization 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 fully executed and onboard analysis is complete.

Cell morphology detection uses a combination of reversible fluorescent dyes and organelle-specific probes coupled to fluorescent reporters. Fluorescence signals are imaged and analyzed to identify cellular structures and extract morphology features.

Optimization Run Consumables

A cell culture optimization run on the AVITI24 System requires **one** of each of the following Cytos consumables:

- Cytos Onboard Optimization Kit
- Cytos Slide Kit (48-well or 12-well or 1-well)
- AVITI24 Flow Cell Assembly Kit

The following reusable tools are also required:

- Flow Cell Assembly Tool Set
- Cytos Centrifuge Adapter

For a list of kits with catalog numbers, see [Consumables and Tools on page 48](#).

Cytos Onboard Optimization Kit

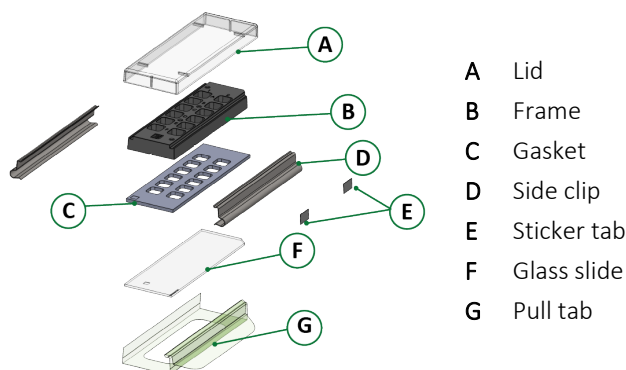
The Cytos Onboard Optimization Kit includes a Cytos Optimization Cartridge and buffer bottle. The Cytos Optimization Cartridge is used for cell culture optimization run on an AVITI24 System and includes required reagents for one run.

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.

Surface area of each slide kit:

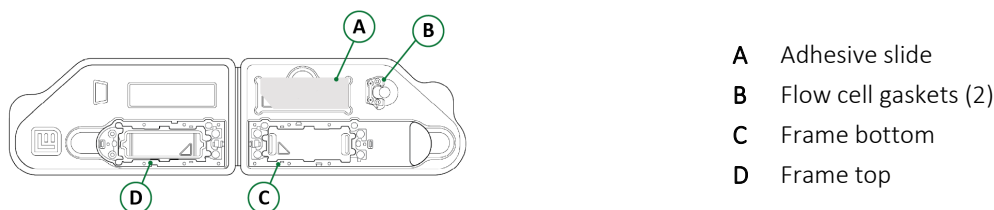
- 48-Well Slide Kit: $\sim 0.12 \text{ cm}^2/\text{well}$
- 12-Well Slide Kit: $\sim 0.5 \text{ cm}^2/\text{well}$
- 1-Well Slide Kit: $\sim 10 \text{ cm}^2/\text{well}$



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 on page 11](#).

AVITI24 Flow Cell Assembly Kit

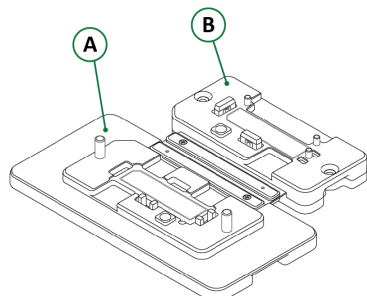
The AVITI24 Flow Cell Assembly Kit contains an adhesive slide, two flow cell gaskets, and the top and bottom frame. Before the run, the sample slide is assembled and packaged into a Cytos flow cell using the provided components. Based on the slide kit that you are using, select the appropriate AVITI24 Flow Cell Assembly Kit. See [Assemble the Cytos Flow Cell on page 34](#).



Flow Cell Assembly Tool Set

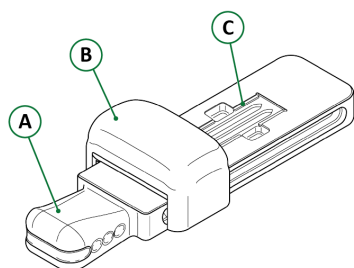
The Flow Cell Assembly Tool Set includes tools to convert the Cytos Slide Kit into a flow cell using parts provided in the AVITI24 Flow Cell Assembly Kit.

- **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 35](#).



- A Side labeled **Sample** for sample slide
- B Side labeled **Adhesive** for adhesive slide

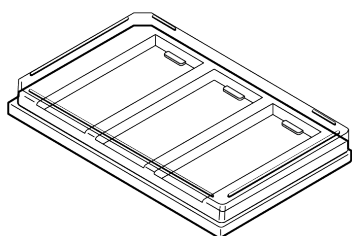
- **Flow Cell Sealer**—The flow cell sealer ensures a secure seal of the two affixed slides as you *slowly* move the roller grip forward and back. For care instructions, see [Maintain the Flow Cell Sealer on page 50](#).



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

Cytos Centrifuge Adapter

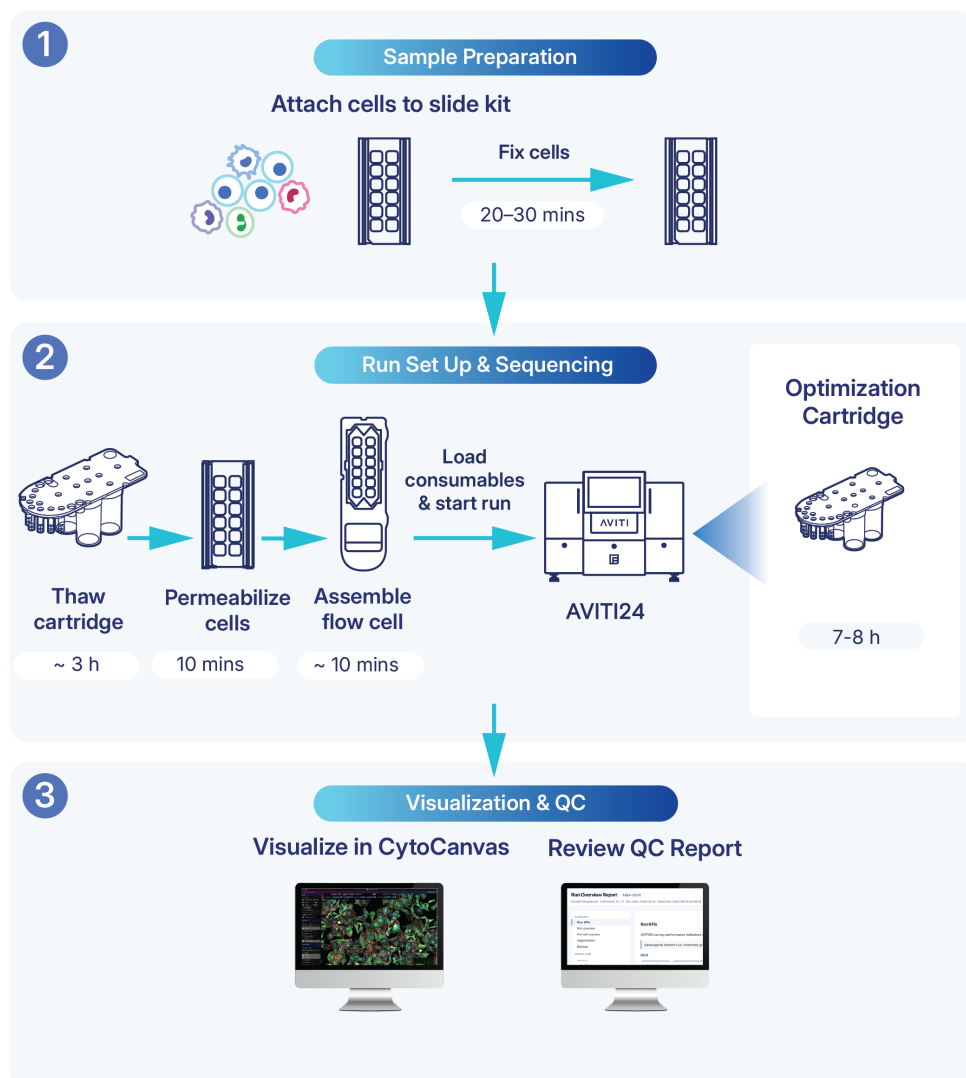
The Cytos Centrifuge Adapter allows for centrifugation of Cytos Slide Kits. The adapter size matches the dimensions of a Society for Biomolecular Screening (SBS) standard 96-well microplate and fits most tabletop laboratory centrifuges. Each Cytos Centrifuge Adapter can hold up to three Cytos Slide Kits.



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

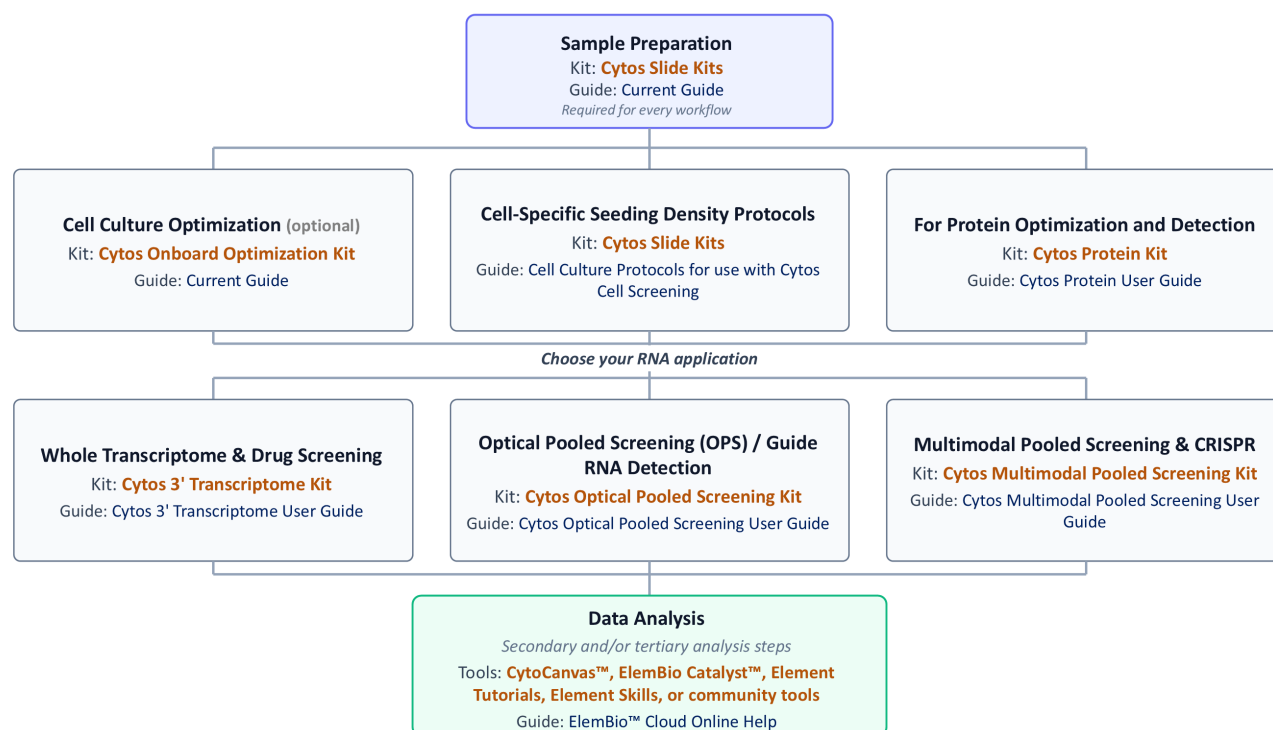
The following materials are required for sample preparation and performing a Cytos optimization run. For details and storage conditions, see [Consumables and Tools on page 48](#). For details and supplier information on materials supplied by the user, see [User-Supplied Consumables on page 48](#).

Materials	Included with a Kit / User Supplied
Cytos Slide Kit with fixed cells	See Sample Preparation on page 21 .
For cell culture	
Cytos Slide Kit, Uncoated	Included in Cytos Slide Kit
Cytos Centrifuge Adapter	Available for purchase
Healthy Cells (freshly dissociated)	User Supplied
Cell culture medium appropriate for the cell line	User Supplied
Preferred surface coating for slide kit	User Supplied
1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium and magnesium	User Supplied
Microseal 'B' adhesive seals, or equivalent	User Supplied
RiboLock RNase Inhibitor	User Supplied
96-well square glass-bottom plate or 384-well square glass-bottom plate (for seeding density optimization)	User Supplied
Pipettes (8-channel or 16-channel for 48-well slide kits or 8-channel for 12-well slide kits or single for 1-well slide kits)	User Supplied
For Fixation	
Formaldehyde (37% by weight, molecular biology)	User Supplied
For optimization run	
Cytos Onboard Optimization Kit	Included with the kit
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

Supported Protocols

Different Cytos user guides document the complete end-to-end workflow, from sample preparation through performing a cell screening run with a specific cartridge. Use the application specific user guide to ensure you are using the correct protocol.

This guide documents the generic sample preparation protocols and details for using the Cytos Optimization Cartridge for an optimization run. See the following chart and table to follow a different protocol and use the applicable user guide and kits.



Application	Kits	User Guide
Generic Sample Preparation protocols and guidelines	Cytos Slide Kits	Current guide
Onboard Optimization	Cytos Onboard Optimization Kit Cytos Slide Kits	Current guide
Cell-specific seeding density protocols	Cytos Slide Kits	Cell Culture Protocols for use with Cytos Cell Screening
Protein optimization and detection	Cytos Protein Kit	Cytos Protein User Guide (MA-00095)
Optical Pooled Screening (OPS)/guide RNA detection	Cytos Optical Pooled Screen Kit	Cytos Optical Pooled Screening User Guide (MA-00096)
Whole transcriptome screening, drug screening	Cytos 3' Transcriptome Kit	Cytos 3' Transcriptome User Guide (MA-00098)
Multimodal Pooled Screening and CRISPR	Cytos Multimodal Pooled Screening Kit	Cytos Multimodal Pooled Screening User Guide (MA-00097)
Data Analysis including secondary and tertiary data analysis steps	Using tools such as CytoCanvas, ElemBio Catalyst, Element Tutorials, Element Skills or community tools	ElemBio Cloud Online Help

Custom Surface Coatings

Uncoated Cytos slide kits are available for preparing a user-applied custom coating. Applying a coating increases cell adhesion and avoids cell loss during washing and other assay steps. Certain cell types require an extracellular matrix for proper cell adhesion and growth. Some cells show better results when a second coating of an extracellular matrix such as collagen, fibronectin, or matrigel is applied on top of a Poly-L-Lysine (PLL)-coated slide.



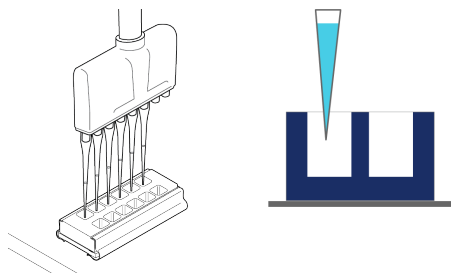
NOTE

Be sure to coat the Cytos slides with PLL or a custom coating before cell culture to minimize cell loss.

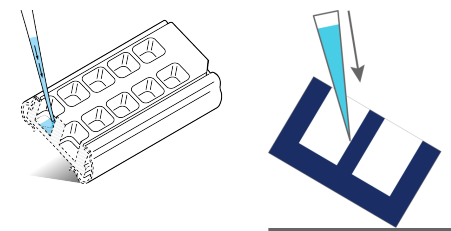
Perform all steps in a biosafety cabinet.

Pipette Placement Guidance

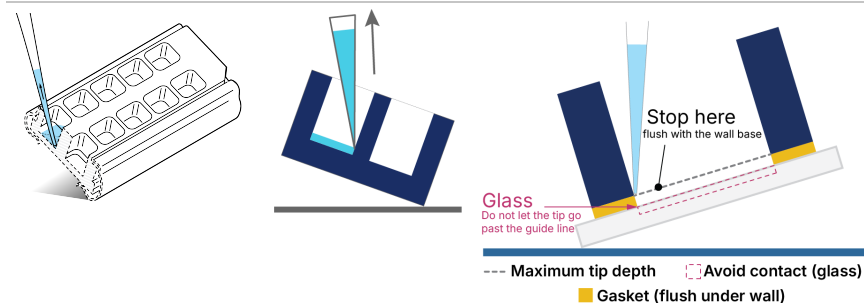
Ensure proper pipette placement during on-flow cell treatments as follows:



- When dispensing cells, hold the pipette perpendicular to the glass slide surface and dispense into the center of each well.
- Do not contact the slide surface.
- Do not swirl the pipette when loading.
- To reduce performance variability, ensure the slide kit is on a flat and level surface during cell seeding.
- Dispense smoothly and avoid dropwise distribution.



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



- 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.

Slide Preparation

Regardless of your preferred custom coating, the slide preparation steps are the same. Use the following instructions to prepare the slide and then proceed to the preferred surface coating instructions.

1. Gather the following consumables:
 - » 0.1 N NaOH solution
 - » Biological-grade/RNase-free water
 - » Cytos Slide Kit
2. Remove the Cytos Slide Kit from the package. Gently press along the pull tab to ensure it is securely adhered to the metal clip.
3. Add the appropriate volume of 0.1 N NaOH solution to each well. Make sure the entire surface in each well is covered.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml



NOTE

For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpipette. See [User-Supplied Consumables on page 48](#).

4. **For the 48-well slide kit only**—Gently tap the slide kit frame a few times on both sides and visually inspect for bubbles.
 - » Make sure that no bubbles are trapped in the bottom of the wells.
 - » Centrifuge the slide kit at 130 x g for 2.5 minutes. Balance the centrifuge with another slide kit tray.
5. Incubate at **room temperature for 15 minutes** inside the biosafety cabinet.
6. Remove the solution from each well.
7. Wash each well with the appropriate volume of biological-grade/RNase-free water.

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

8. Repeat the wash **5 more times**. Remove the final wash solution from each well.
9. Proceed to the preparation steps for your preferred surface coating:
 - » [Collagen Coating on page 13](#)
 - » [Fibronectin Coating on page 13](#)
 - » [Gelatin Coating on page 14](#)
 - » [Laminin Coating on page 14](#)
 - » [Matrigel Coating on page 15](#)
 - » [Poly-L-Lysine \(PLL\) Coating on page 16](#)

Collagen Coating

1. Make sure [Slide Preparation](#) is complete.
2. Gather the following consumables:
 - » Collagen Type 1 stock solution—Store at 2°C to 8°C (MilliporeSigma, catalog # C3867-1VL)
 - » Hydrochloric acid (HCl), 0.01 N
 - » Biological-grade/RNase-free water
3. If the collagen stock solution appears thick, set aside at room temperature for 15–30 minutes. Do not exceed 2 hours.
4. Dilute the collagen I stock solution to a concentration of 20 µg/ml with 0.01 N HCl.
5. Add the appropriate volume of diluted collagen solution to each well. Make sure the entire surface in each well is covered.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml

6. Incubate at **room temperature for 1 hour** inside the biosafety cabinet.
7. Carefully pipette along the wall of each well to remove remaining solution without disturbing the slide surface.
8. Wash each well with the appropriate volume of biological-grade/RNase-free water.

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

9. Repeat the wash **two more times**. Pipette along the wall of each well to *completely* remove the final wash solution.
10. Allow the surface to **air-dry for 15 minutes**.
11. Seal the wells with an adhesive seal and store the slide dry at 2°C to 8°C for up to 10 days. Do not use if surface cracking exists.

Fibronectin Coating

1. Make sure [Slide Preparation](#) is complete.
2. Gather the following consumables:
 - » Fibronectin stock solution—Store at 2°C to 8°C (MilliporeSigma, catalog # F1141-2MG)
 - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
 - » Biological-grade/RNase-free water
3. Dissolve and dilute the fibronectin stock solution to a final concentration of 2 µg/ml in 1X PBS.
4. Add the appropriate volume of diluted fibronectin solution to each well. Make sure the entire surface in each well is covered.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml

5. Incubate at **room temperature for 1 hour** inside the biosafety cabinet.
6. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
7. Wash each well with the appropriate volume of biological-grade/RNase-free water.

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

8. Repeat the wash **two more times**. Pipette along the wall of each well to *completely* remove the final wash solution.
9. Allow the surface to **air-dry for 15 minutes**.

10. Seal the wells with an adhesive seal and store the slide dry at 2°C to 8°C for up to 10 days. Do not use if surface cracking exists.

Gelatin Coating

1. Make sure [Slide Preparation](#) is complete.
2. Gather the following consumables:
 - » Gelatin solution, Type B, 2% in H₂O—Store at 2°C to 8°C (MilliporeSigma, catalog # G1393-20ML)
 - » Biological-grade/RNase-free water
3. If the gelatin stock solution is cloudy, place the gelatin in a 37°C water bath for 1 hour or until the solution is clear. Do not exceed 2 hours.
4. Dissolve and dilute the gelatin stock solution to a final concentration of 50 µg/ml in biological-grade/RNase-free water.
5. Add the appropriate volume of diluted gelatin solution to each well. Tap or swirl the slide kit to ensure coverage in each well.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	100 µl	1.25 ml

6. Incubate at **room temperature for 1 hour** inside the biosafety cabinet.
7. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
8. Wash each well with the appropriate volume of biological-grade/RNase-free water.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml

9. Repeat the wash **two more times**. Pipette along the wall of each well to remove the final wash solution.
10. Add the appropriate volume of biological-grade/RNase-free water and seal the wells with an adhesive seal. **Do not allow the surface to dry.**

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml

11. Store the prepared slide at 2°C to 8°C for up to 10 days. Do not use if discoloration or surface cracking exists.

Laminin Coating

1. Make sure [Slide Preparation](#) is complete.
2. Gather the following consumables:
 - » Laminin stock solution—Store at -85°C to -75°C (Gibco Laminin Mouse Protein, Natural, catalog # 23017-015)
 - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
3. Thaw laminin at 2°C to 8°C for 15–30 minutes. Do not exceed 1 hour.
 - » Avoid rapid warming of laminin, which causes laminin to form a gel and prevents further use.
 - » Avoid multiple thaw cycles. Store small quantities of laminin at -25°C to -15°C for up to 6 months.
 - » Place the laminin in an ice bucket when handling at room temperature.
4. Dissolve and dilute laminin stock solution to a final concentration of 50 µg/ml in 1X PBS.
5. Add the appropriate volume of diluted laminin solution to each well. Tap or swirl the slide kit to ensure coverage in each well.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
30 µl	100 µl	1.25 ml

6. Incubate at **room temperature for 1 hour** inside the biosafety cabinet.
7. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
8. Wash each well with the appropriate volume of 1X PBS.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 μ l	150 μ l	2 ml

9. Repeat the wash **two more times**.
10. Add the appropriate volume of 1X PBS and seal the wells with an adhesive seal. **Do not allow the surface to dry.**

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 μ l	150 μ l	2 ml

11. Store the slide at 2°C to 8°C for up to 10 days. Do not use if discoloration or surface cracking exists.

 **NOTE** Laminin contains a wheat germ agglutinin (WGA) binding site that can reduce the efficacy of cell segmentation. If this surface coating is required for cell culture, use Cytos Protein Alternative Morphology Panel for segmentation.

Matrigel Coating

You may need to optimize the matrigel used for surface coating as the matrigel concentration depends on your cell line and the type of matrigel used for coating. Element recommends using a range of 0.05–1 mg/ml matrigel concentration. Ensure the matrigel concentration is < 2.5 mg/ml to retain an even coating and reduce background staining.

1. Make sure [Slide Preparation](#) is complete.
2. Gather the following consumables:
 - » Matrigel stock solution—Store at -25°C to -15°C (Corning Matrigel Basement Membrane Matrix, catalog # 356237 or Corning Matrigel Growth Factor Reduced Basement Membrane Matrix, catalog # 356231)

 **NOTE** Be sure to use Matrigel that is phenol-red free.

- » 1X Phosphate Buffered Saline (PBS), pH 7–7.4, chilled
3. Thaw matrigel according to manufacturer's instructions.
 - » Avoid multiple thaw cycles. Store small quantities of matrigel at -25°C to -15°C.
 - » When thawed, swirl the vial of matrigel to ensure all the material is dispersed.
4. Once matrigel is thawed, place matrigel and 1X PBS on ice and place the ice bucket with the matrigel and 1X PBS in the refrigerator for 1 hour. Chill the slide kit at 2°C to 8°C for 1 hour.
5. Dissolve and dilute matrigel stock solution to a final concentration in chilled 1X PBS.
Place the matrigel in an ice bucket when handling at room temperature.
6. Place the slide kit on ice to help spread the matrigel solution in the wells in the next step.
7. Add the appropriate volume of diluted matrigel solution to each well. Tap or swirl the slide kit to ensure coverage in each well.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 μ l	100 μ l	1.25 ml

8. Incubate at **room temperature for 1 hour** inside the biosafety cabinet.
9. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.

10. Add the appropriate volume of 1X PBS and seal the wells with an adhesive seal. **Do not allow the surface to dry.**

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml

11. Store the slide at 2°C to 8°C for up to 10 days. Do not use if discoloration or surface cracking exists.

 **NOTE** Matrigel contains a wheat germ agglutinin (WGA) binding site that can reduce the efficacy of cell segmentation. If this surface coating is required for cell culture, use Cytos Protein Alternative Morphology Panel for segmentation.

Poly-L-Lysine (PLL) Coating

1. Make sure [Slide Preparation](#) is complete.
2. Gather the following consumables:
 - » PLL stock solution, 0.01%—Store at 2°C to 8°C (MilliporeSigma, catalog # P4707-50ML)
 - » Biological-grade/RNase-free water
3. Add the appropriate volume of 0.01% PLL solution to each well. Make sure the entire surface in each well is covered.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml

4. Incubate at **room temperature for 15 minutes** inside the biosafety cabinet.
5. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
6. Wash each well with the appropriate volume of biological-grade/RNase-free water.

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

7. Repeat the wash **two more times**. Vacuum aspirate along the wall of each well to *completely* remove the final wash solution.
8. Allow the surface to **air-dry for 15 minutes**.
9. Seal the wells with an adhesive seal and store the slide dry at 2°C to 8°C for up to 10 days.

Double Surface Coating with Poly-L-Lysine and Matrigel

1. Make sure [Slide Preparation](#) is complete.
2. Gather the following consumables:
 - » PLL stock solution, 0.01%—Store at 2°C to 8°C (MilliporeSigma, catalog # P4707-50ML)
 - » Biological-grade/RNase-free water
 - » Matrigel stock solution—Store at -25°C to -15°C (Corning Matrigel Basement Membrane Matrix, catalog # 356237 or Corning Matrigel Growth Factor Reduced Basement Membrane Matrix, catalog # 356231)

 **NOTE** Be sure to use Matrigel that is phenol-red free.

- » 1X Phosphate Buffered Saline (PBS), pH 7–7.4, chilled
3. Thaw matrigel according to manufacturer's instructions.
 - » Avoid multiple thaw cycles. Store small quantities of matrigel at -25°C to -15°C.
 - » When thawed, swirl the vial of matrigel to ensure all the material is dispersed.

4. Once matrigel is thawed, place matrigel and 1X PBS on ice and place the ice bucket with the matrigel and 1X PBS in the refrigerator for 1 hour.
5. Add the appropriate volume of 0.01% PLL solution to each well. Make sure the entire surface in each well is covered.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 μ l	150 μ l	2 ml

6. Incubate at **room temperature for 15 minutes** inside the biosafety cabinet.
7. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
8. Wash each well with the appropriate volume of biological-grade/RNase-free water.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
50 μ l	200 μ l	3 ml

9. Repeat the wash **three more times**. Vacuum aspirate along the wall of each well to *completely* remove the final wash solution.
10. Allow the surface to **air-dry for 15 minutes** ensuring there are no droplets left at the edge of the well.
11. Immediately place the slide kit in a Petri dish and seal the Petri dish. Place the Petri dish with the slide kit at 2°C to 8°C for 30 minutes.
12. Place the matrigel and 1X PBS in an ice bucket when handling at room temperature.
13. Dissolve and dilute matrigel stock solution to a final concentration in chilled 1X PBS.
Element recommends using a range of 0.05–1 mg/ml matrigel concentration.
14. Place the slide kit on ice to help spread the matrigel solution in the wells in the next step.
15. Add the appropriate volume of diluted matrigel solution to each well. Tap or swirl the slide kit to ensure coverage in each well.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 μ l	100 μ l	1.25 ml

16. Incubate at **room temperature for 1–2 hours** inside the biosafety cabinet.
17. Carefully pipette along the wall of each well to remove excess solution without disturbing the slide surface.
18. Add the appropriate volume of 1X PBS and seal the wells with an adhesive seal. **Do not allow the surface to dry.**

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 μ l	150 μ l	2 ml

19. Store the slide at 2°C to 8°C for up to 10 days. Do not use if discoloration or surface cracking exists.

 **NOTE** Matrigel contains a wheat germ agglutinin (WGA) binding site that can reduce the efficacy of cell segmentation. If this surface coating is required for cell culture, use Cytos Protein Alternative Morphology Panel for segmentation.

Seeding Density Optimization

If you have optimized and identified an ideal seeding density for your cell line for the Cytos assay, proceed to preparing samples. See [Sample Preparation on page 21](#).

To identify the optimal seeding density for a specific cell type for a Cytos assay, perform a cell density titration on a 12-well Cytos Slide Kit or 48-well Cytos Slide Kit. This section provides guidelines on seeding density and cell culture optimization for adherent cells and suspension cells using Cytos Slide Kits and bright field microscopy.

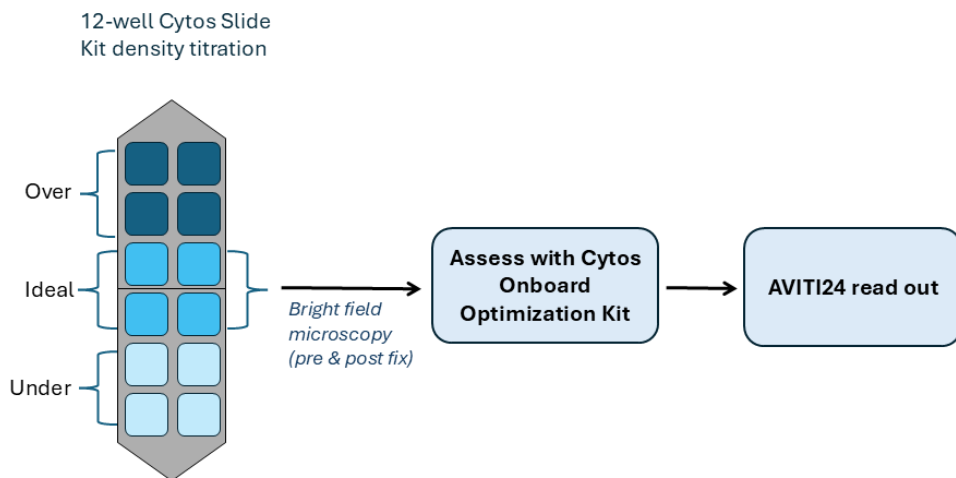
If additional cell culture or surface coating optimization is required, you can use 96-well or 384-well glass plates to assess cell growth and health. See [Additional Optimization on page 52](#).

Guidelines and Workflow

- **Perform cell culture steps in a biosafety cabinet.**
- Perform optimization experiments in replicates and randomize the sample setup to minimize any performance bias due to well positions on the slide kit.
- Depending on your specific cell line, you can coat an uncoated Cytos slide with commonly used surface coating reagents and assess efficacy of these different coating reagents. See [Custom Surface Coatings on page 11](#).
- Use cells cultured on a Cytos slide at a desired confluency. For example, optimal confluency for HeLa cells is 50–70%. Over- or under-confluency can result in segmentation errors or reduce overall data yield.
- Ensure proper pipette placement as described in the cell culture sections. See [Protocol Guidelines on page 21](#).

Seeding Density Optimization Workflow

The following workflow shows a density titration with a 12-well Cytos Slide Kit. The cells were imaged before and after sample fixation using bright field microscopy. The cells at ideal confluency are then assessed with Cytos Onboard Optimization Kit and imaged on an AVITI24 System. A similar density titration can also be performed with a 48-well Cytos Slide Kit.



Using Cytos Slide Kit

When working with new cell lines, Element recommends performing a cell density titration on a Cytos Slide Kit for rapid assessment of seeding densities to quickly identify an initial compatible cell density range for a Cytos assay. A seeding density titration is performed on the appropriate Cytos Slide Kit at varying cell concentrations to include a broad range of cell densities to identify a suitable starting density for the Cytos assay. Following the appropriate growth period, assess cells using bright field microscopy and identify optimal seeding density that achieves 50–70% target confluency for the cell line.

The following sections provide detailed examples of performing cell density titrations for adherent and suspension cells on a Cytos Slide Kit.

Culture and fix adherent cells

Seed freshly dissociated cells at varying concentrations onto a coated Cytos Slide Kit for growth and proliferation. Fix cells with 4% formaldehyde. See [Culture and Fix Adherent Cells on page 22](#). View cells using a bright field microscope to identify the optimal seeding density. See [Assess Cell Sample Using Bright Field Microscopy on page 20](#).

Use the following table as an example of an experimental setup for a 12-Well and 48-Well Cytos Slide Kit with varying seeding densities for adherent **HeLa** cells. You can also use the surface area of the Cytos Slide kits to determine initial seeding densities for your specific cell type. See [Cytos Slide Kit on page 6](#).

Well	48-Well Slide Kit (Cells/Well)	12-Well Slide Kit (Cells/Well)
A	1000	3000
B	2000	5000
C	3000	9000
D	4000	10,000
E	6000	18,000
F	7000	20,000

Culture and fix suspension cells

Attach live suspension cells at varying concentrations onto a coated Cytos Slide Kit using centrifugation. Fix cells with 4% formaldehyde. See [Attach and Fix Suspension Cells on page 25](#). View cells using a bright field microscope to identify the optimal seeding density. See [Assess Cell Sample Using Bright Field Microscopy on page 20](#).

Use the following table as an example of an experimental setup for a 12-Well and 48-Well Cytos Slide Kit with varying seeding densities for suspension **Jurkat** cells. You can also use the surface area of the Cytos Slide kits to determine initial seeding densities for your specific cell type. See [Cytos Slide Kit on page 6](#).

Well	48-Well Slide Kit (Cells/Well)	12-Well Slide Kit (Cells/Well)
A	10,000	30,000
B	15,000	60,000
C	20,000	90,000
D	35,000	150,000
E	50,000	175,000
F	75,000	200,000

Assess Cell Sample Using Bright Field Microscopy

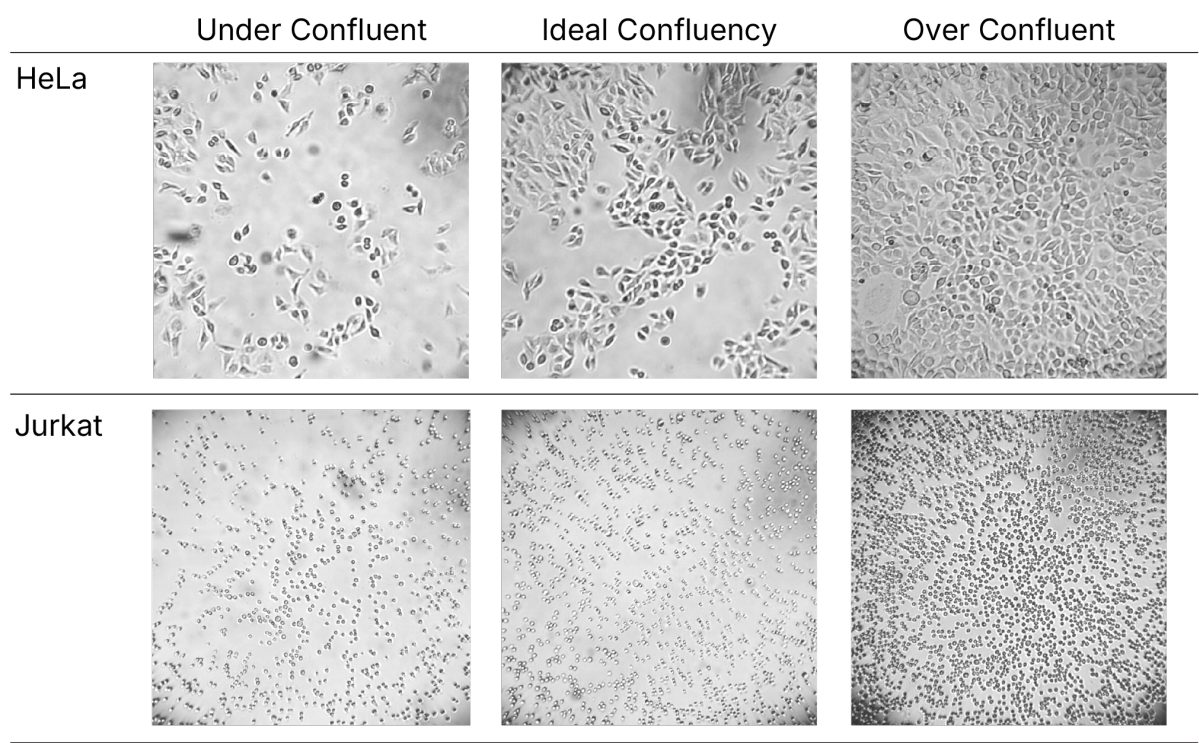
Use a standard laboratory bright field microscope to view cell samples and assess cell confluency. Generic instructions for viewing cell samples using an inverted microscope are included in this section. For further details, follow the user manual supplied with the microscope.

1. Place the Cytos Slide Kit or glass plate with cells securely on the microscope stage.
2. Scan the slide kit or plate using the lowest magnification to easily locate cells.
3. Adjust the brightness and intensity to clearly view cells.
4. Perform fine adjustments using the focus knob until cells appear sharp.
5. After locating and adjusting the cell image, you can switch to higher magnification if needed.
6. View the cell confluency and cell health. Record the final confluency before and after fixation.

Visit the [Cell Culture Hub](#) to view bright field microscopy images of seeding density experiments for a variety of cell lines. Reference the hub images to compare and assess if cells are under or over confluent, or at the ideal confluency.

7. After reviewing bright field microscopy results, proceed to one of the following options:
 - » Repeat the density titration with extended seeding density range to identify an optimal range if results show under or over confluency for your cell type.
 - » If results show ideal confluency for at least a few wells, assess sample preparation using Cytos Onboard Optimization Kit. See [Run Preparation and Setup on page 29](#).
 - » If further optimization is required, use glass plates to test various seeding densities and surface coatings. See [Additional Optimization on page 52](#).

The following example shows seeding density titration results on a 48-well Cytos Slide Kit for HeLa and Jurkat cells, and viewed post fixation using an OMAX K832 inverted bright field microscope at 4X magnification.



See [Troubleshoot Sample Preparation on page 44](#) for any sample preparation issues.

Sample Preparation

Sample preparation on a Cytos Slide Kit is required for all Cytos runs on an AVITI24 System. This section describes sample preparation for adherent and suspension cells.

- [Culture and Fix Adherent Cells on page 22](#)
- [Attach and Fix Suspension Cells on page 25](#)

Optimize seeding densities and cell culture conditions for your cell line using Cytos Slide Kits and bright field microscopy. To assess sample preparation quality, use the Cytos Onboard Optimization Kit (part # 860-00056) to view samples after a short run on the AVITI24 System. See [Seeding Density Optimization on page 18](#). Reference the [Cell Culture Hub](#) for cell images and confluency.



NOTE

Cells expressing fluorescent proteins can interfere with Cytos readouts and are not recommended.

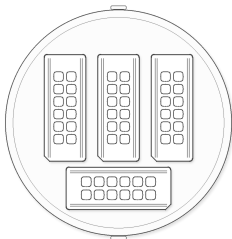
Protocol Guidelines



IMPORTANT

Ensure proper pipette placement during on-flow cell treatments to minimize touching the glass surface or disturbing the cell layer. See [Pipette Placement Guidance on page 11](#).

- **Perform sample preparation steps in a biosafety cabinet.** Steps involving live cells *must* be performed in a biosafety cabinet.
- Use healthy and actively dividing cells that exhibit > 85% viability with trypan blue staining.
- Avoid disturbing the slide surface throughout the protocol.
- Do not allow cells to dry out. Allowing cells to dry out can result in cell detachment.
- To evenly distribute cells on the slide, gently move the covered slide kit for 30 seconds using a forward-and-back, then side-to-side motion. Do not move in a circular motion.
- Use soft/slow deceleration and Brake-off settings if available on your centrifuge to minimize any mechanical stress on cells.
- To prevent cells from lifting or folding due to liquid force and minimize uneven cell distribution, use a half-liquid exchange method for media/reagent exchange steps by retaining a small amount of liquid in the well at each step as described in this user guide.
- Place the slide kits inside a 10 cm Petri dish during all handling protocols. Up to four slide kits can be placed inside a 10 cm Petri dish.



- When placing the Petri dish with slide kits in the incubator, ensure that the incubator racks are at level and avoid stacking multiple Petri dishes on top of each other.

Culture and Fix Adherent Cells

Sample preparation of adherent cells involves steps to culture cells on the Cytos Slide Kit and then fix cultured cells.

- **Culture**—Seeds freshly dissociated cells onto a treated surface for growth and proliferation, resulting in a consistent cell layer.
- **Fix**—Binds cells to the slide while halting cell function and preserving the structure of the bound cells.

Refer to [Cell Culture Protocols for use with Cytos Cell Screening](#) for detailed adherent cell-specific protocols that include seeding density, surface coatings, and images showing successful cell culture techniques.

Culture Adherent Cells

1. Gather the following consumables:
 - » Cell culture medium appropriate for the cell line
 - » Cytos Slide Kit coated with a surface coating, equilibrated to room temperature
 - » Reagent trough
 - » [Optional] Plate rocker (VWR Scientific Platform Rocker, catalog # 444-0927) or shaker (Qinstruments Bioshake XP, catalog # 1808-0505)
2. Warm the cell culture medium in a 37°C water bath.
3. If your slide kit with custom surface coating was stored in liquid, remove any liquid in the wells.
4. Wash each well with the appropriate volume of prewarmed culture medium. Slightly tip the slide kit and slowly dispense along the wall of each well. Do not contact the slide surface. Ensure the media covers the surface in each well.

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



NOTE

For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpiptette. See [User-Supplied Consumables on page 48](#).

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. Repeat the wash **one more time**.
7. Ensure the cells are fully dissociated to single cells and counted.
8. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently dispense the appropriate volume of suspended cells in the center of the well. Do not swirl the pipette when loading. Do not contact the slide surface.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	2 ml

Use the seeding density listed for your specific cell type in the [cell-specific protocol](#) or use the following information to estimate initial seeding density.

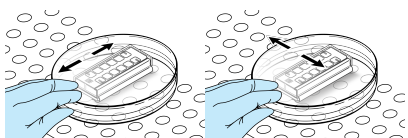
	48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
Well size:	3.5 mm x 3.5 mm	7 mm x 7 mm	54 mm x 19 mm
Element uses:	2.4–2.8 K HeLa cells per well	9–10 K HeLa cells per well	120–180 K HeLa cells per well

9. Cover the slide kit. Distribute the cells manually or use a plate rocker or plate shaker as follows:

Manual Cell Distribution

- » Gently distribute the cells for 30 seconds using a forward-and-back, pause for 5 seconds, and then side-to-side motion.

- » At a slow pace, move forward and back and side to side covering at least 5 inches (12.5 cm) in each direction.
- » *Do not move in a circular motion.* Do not allow the liquid to splash within the wells.



Using a Plate Rocker or Plate Shaker to Distribute Cells

- » Place the slide kit in a Cytos Centrifuge Adapter (part # 860-00069).
 - » Set the adapter with slide kit on the shaker or rocker as follows:
 - Set the plate rocker at speed set at 2 and rock the slide kit for 2.5 minutes for cell distribution.
 - Set the plate shaker at speed set at 200 for 2.5 minutes for cell distribution.
 - Rotate the slide kit to 90° at ~ 1 minute to ensure proper cell distribution in all directions.
10. **For the 48-well slide kit only**—Place the 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.
 11. Incubate the cells at **37°C** to target ideal confluency of 50–70% in each well. Do not allow cell overgrowth. As an example, Element incubates HeLa cells for 16 to 18 hours.

Fix Cultured Adherent Cells

Do not prepare more than three 12-well Cytos Slide Kits or one 48-well Cytos Slide Kit at the same time to prevent cells from drying out during the fixation process.

Fixation of adherent cells involves the following steps:

- **Wash with 1X DPBS**—Wash each well with warm 1X DPBS four times to remove dead cells using half-liquid exchange method.
- **Fix Cells**—Add 8% Fixation Reagent to wells containing 1X DPBS using half-liquid exchange method.
- **Wash with 1X PBS**—Wash each well with 1X PBS three times to remove the Fixation Reagent.



IMPORTANT

Fixation process follows the half-liquid exchange method by retaining a small amount of liquid in the well at each step to minimize uneven cell distribution.

1. Gather the following consumables:
 - » 1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, pH 7–7.4, sterilized (ThermoFisher Scientific, catalog # 14040117)
 - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
 - » Freshly prepared Formaldehyde, 8% (Fixation reagent), dilute as needed with DPBS
2. Warm the 1X DPBS in a 37°C water bath.
3. Retrieve the slide kit containing cultured cells. Do not remove the medium.
4. Use the steps in the following table to wash and fix cells using the half-liquid exchange method.

To remove liquid, slightly tip the slide kit and position the pipette tip in the well corner without contacting the slide surface.

To add liquid, slightly tip the slide kit and slowly dispense along the wall of each well.

Step	48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
Add prewarmed 1X DPBS	40 µl	150 µl	2 ml
Remove medium/DPBS mix	40 µl	150 µl	2 ml
Repeat wash with prewarmed 1X DPBS three times using half-liquid exchange method	40 µl	150 µl	2 ml
Remove 1X DPBS	40 µl	150 µl	2 ml
Add 8% Fixation Reagent below the liquid level to ensure mixing within the well	40 µl	150 µl	2 ml

5. With a lid on the slide kit, incubate at **room temperature** for **20–30 minutes**. Do not exceed 30 minutes.
6. To remove the fixation reagent, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
7. Wash each well with the appropriate volume of *1X PBS*.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	200 µl	2 ml

8. 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.
9. Repeat the wash **two more times**. Do not remove the liquid after the final wash.
10. View cells using a bright field microscope to ensure there is no cell loss after the fixation protocol.
11. After fixing cells, proceed to one of the following options:
 - » Assess success of sample preparation. See [Run Preparation and Setup on page 29](#).
 - » Perform a cell screening run on the AVITI24 System using a specific cartridge. See [Supported Protocols on page 10](#).
 - » Store samples for future use, see [Prepare Cell Samples for Storage on page 28](#).
 - » If you plan to ship samples, see [Shipping Samples on page 51](#).

Attach and Fix Suspension Cells

Sample preparation of suspension cells involves steps to attach cells to the Cytos Slide Kit and then fix attached cells.

- **Attach**—Attaches and immobilizes live suspension cells to a treated surface using centrifugation.
- **Fix**—Crosslinks cells to the slide while halting cell function and preserving the structure of the bound cells.

Refer to [Cell Culture Protocols for use with Cytos Cell Screening](#) for detailed suspension cell-specific protocols that include seeding density, surface coatings, and images showing successful cell culture techniques.

Attach Suspension Cells on a 48-Well or 12-Well Slide Kit

1. Gather the following consumables:
 - » 1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, pH 7–7.4
 - » Cytos Slide Kit coated with a surface coating, equilibrated to room temperature
 - » 15 ml or 50 ml Falcon tube
 - » Reagent trough
2. Warm the DPBS in a 37°C water bath.
3. To ensure DPBS remains sterile, always open the DPBS bottle inside the biosafety cabinet.
4. Centrifuge the cells for **5 minutes at 300 x g** in a 15 ml or 50 ml Falcon tube depending on final volume.
5. Remove the supernatant without disturbing the cell pellet.
6. Add 1X DPBS to resuspend the cell pellet and dilute the cell solution depending on desired confluency.
7. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently dispense the appropriate volume of cell solution in the center of the well. Do not swirl the pipette when loading. Do not contact the slide surface.

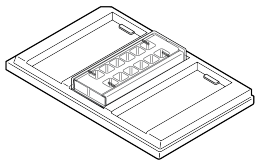
48-Well Slide Kit	12-Well Slide Kit
40 µl	150 µl

 **NOTE** For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpiptette. See [User-Supplied Consumables on page 48](#).

Use the seeding density listed for your specific cell type in the [cell-specific protocol](#) or use the following information to estimate initial seeding density.

	48-Well Slide Kit	12-Well Slide Kit
Well size:	3.5 mm x 3.5 mm	7 mm x 7 mm
Element uses:	28–32 K Jurkat cells per well	100–150 K Jurkat cells per well

8. Cover the wells with the slide kit lid.
9. Load the covered slide kit onto a Cytos Slide Kit Tray (part # 860-00044).
 - » If preparing only one slide kit, load it in the center position.
 - » Balance the centrifuge with another slide kit tray.
 - » If preparing more than one slide kit, divide the slide kits between two slide kit trays.



10. Centrifuge at **300 x g for 15 minutes**.
11. Proceed to [Fix Attached Suspension Cells on page 27](#).

Attach Suspension Cells on a 1-Well Slide Kit

1. Gather the following consumables:
 - » 1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, pH 7–7.4
 - » Slide Kit coated with a surface coating, equilibrated to room temperature
 - » 15 ml Falcon tube
2. Warm the DPBS in a 37°C water bath.
3. To ensure DPBS remains sterile, always open the DPBS bottle inside the biosafety cabinet.
4. Centrifuge the cells for **5 minutes at 300 x g** in a 15 ml or 50 ml Falcon tube depending on final volume.
5. Remove the supernatant without disturbing the cell pellet.
6. Add 5 ml 1X DPBS to resuspend the cell pellet and dilute the cell solution depending on desired confluency.
7. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently add **1.5 ml cell solution**. Do not swirl the pipette. Do not contact the slide surface.

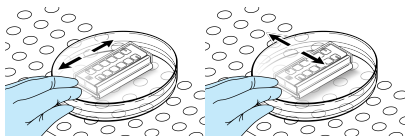
Use the seeding density listed for your specific cell type in the [cell-specific protocol](#) or use the following information to estimate initial seeding density.

Well size: 54 mm x 19 mm	Element uses 1–2 M Jurkat cells per well
--------------------------	--

8. Cover the wells with the slide kit lid. Distribute the cells manually or use a plate rocker or plate shaker as follows:

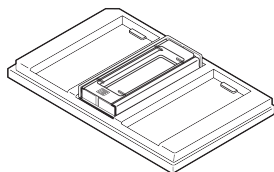
Manual Cell Distribution

- » Gently distribute the cells for 30 seconds using a forward-and-back, pause for 5 seconds, and then side-to-side motion.
- » At a slow pace, move forward and back and side to side covering at least 5 inches (12.5 cm) in each direction.
- » **Do not move in a circular motion. Do not allow the liquid to splash within the wells.**



Using a Plate Rocker or Plate Shaker to Distribute Cells

- » Place the slide kit in a Cytos Centrifuge Adapter (part # 860-00069).
 - » Set the adapter with slide kit on the shaker or rocker as follows:
 - Set the plate rocker at speed set at 2 and rock the slide kit for 2.5 minutes for cell distribution.
 - Set the plate shaker at speed set at 200 for 2.5 minutes for cell distribution.
 - Rotate the slide kit to 90° at ~ 1 minute to ensure proper cell distribution in all directions.
9. Incubate at **room temperature for 15 minutes**.
 10. Load the covered slide kit onto a Cytos Slide Kit Tray (part # 860-00044).
 - » Load only one slide in the center position.
 - » Balance the centrifuge with another slide kit tray.



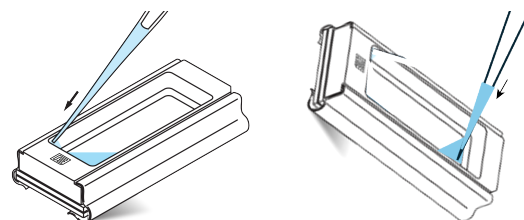
11. Centrifuge at **20 x g for 2 minutes**.

Fix Attached Suspension Cells

Do not prepare more than three 12-well Cytos Slide Kits or one 48-well Cytos Slide Kit at the same time to prevent cells from drying out during the fixation process.

1. Gather the following consumables:
 - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
 - » Freshly prepared Formaldehyde, 8% (Fixation reagent), dilute as needed with DPBS
2. Remove the slide kit tray from the centrifuge, remove the slide kit from the tray, and remove the lid.
3. Do not remove any liquid from the wells.
4. Slightly tip the slide kit and slowly add the appropriate volume of fixation reagent (8% formaldehyde) along the wall of the well in opposite corners. **Do not pipette to mix.**

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	150 µl	750 µl in one corner and 750 µl in opposite corner (total 1.5 ml)



5. Cover the wells with the slide kit lid and incubate at **room temperature for 20–30 minutes**. Do not exceed 30 minutes.
6. To remove the fixation reagent, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
7. Carefully wash each well with the appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the vertical middle of each well wall.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
40 µl	200 µl	2 ml

8. 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.
9. Repeat the wash **two more times**. Do not remove the liquid after the final wash.
10. View cells using a bright field microscope to ensure there is no cell loss after the fixation protocol.
11. After fixing cells, proceed to one of the following options:
 - » Assess success of sample preparation. See [Run Preparation and Setup on page 29](#).
 - » Perform a cell screening run on the AVITI24 System using a specific cartridge. See [Supported Protocols on page 10](#).
 - » Store samples for future use, see [Prepare Cell Samples for Storage on page 28](#).
 - » If you plan to ship samples, see [Shipping Samples on page 51](#).

Prepare Cell Samples for Storage

1. Dilute 40 U/μl RiboLock RNase inhibitor to 0.1 U/μl with 1X PBS.
2. Remove the final wash solution from slide kit wells.
3. Add the appropriate volume of 0.1 U/μl RiboLock RNase inhibitor to each well, covering the surface of each well.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
50 μl	120 μl	1.5 ml

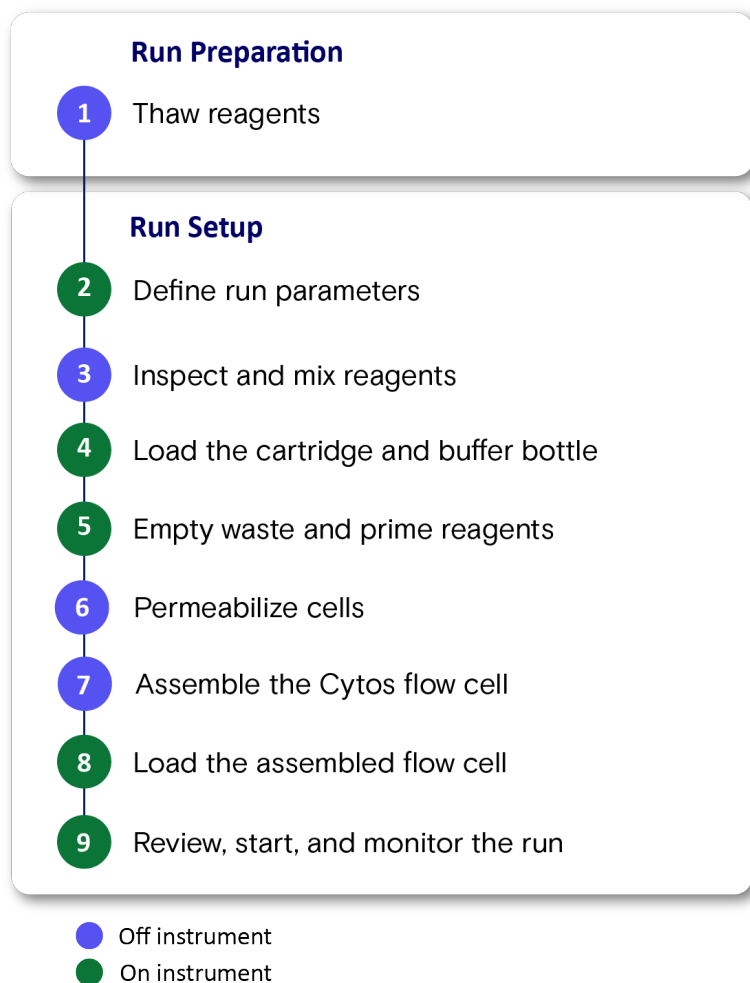
4. Cover the wells with an adhesive seal and store samples at 2°C to 8°C for up to 30 days for most cell lines. Consult Element Technical Support when handling immune cells.

Run Preparation and Setup

Performing cell culture optimization run on an AVITI24 System includes steps to thaw the cartridge, load consumables, assemble the flow cell, and then follow prompts on the AVITI OS interface to set up the run.

Before run preparation and setup, ensure cells are prepared on a Cytos Slide Kit. See [Sample Preparation on page 21](#).

Workflow Summary



Thaw Reagent Cartridge

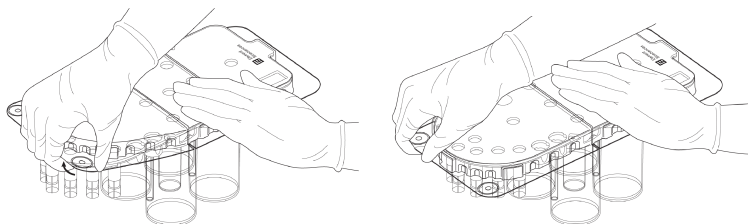
1. Remove a cartridge from -25°C to -15°C storage.



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.**

Set Up an Optimization Cartridge Run

1. If applicable, stage run manifests for import:
 - » If setting up the run manually, save the manifest on a USB and connect the USB drive to an instrument USB port.
 - » Alternatively, you can save the manifest to the specified SMB storage connection.
 - » If you planned the run in ElemBio Cloud, upload the manifest 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 runs on both sides
 - » **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.
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.
6. In the Well Layout field, select **48** or **12** or **1**.
7. Select **Yes** to confirm the Cell Culture Optimization Run option.
8. 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.
9. Proceed to [Inspect and Mix Reagents](#).

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.

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.

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. Confirm each of the consumable preparation steps displayed on the screen and select the checkbox.
4. 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.

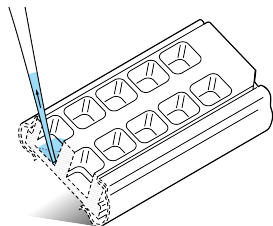
 **NOTE**
Before priming, if you need to discard the run setup, click **Discard** on the top left side of the screen, correct the run setup, and reuse the same cartridge or add a new cartridge as needed. Proceed to the priming step.
8. Select **Next** to *automatically* start priming. Priming takes approximately 27 minutes.
9. While priming is progressing, proceed to [Permeabilize Cells](#).

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.

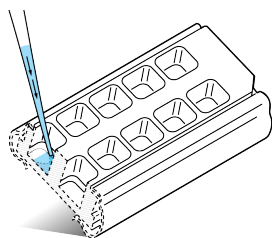
 **NOTE**
See [Pipette Placement Guidance on page 11](#) and [Knowledge Base](#) for detailed pipetting guidance.

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

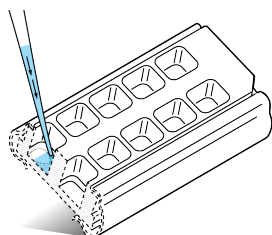


NOTE

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.

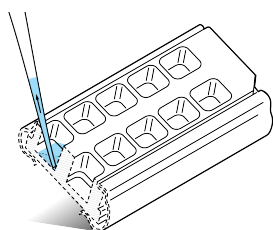
5. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
6. Slightly tip the slide kit and slowly add the appropriate volume of permeabilization reagent along the middle of each well wall.

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 µl tip (recommended)
- Flow cell aligner and flow cell sealer
- AVITI24 Flow Cell Assembly Kit

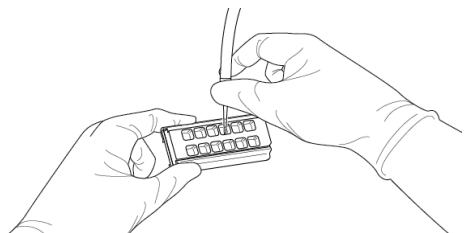


IMPORTANT

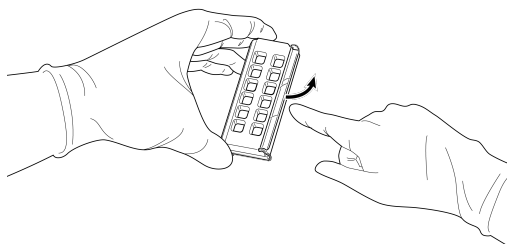
For best results, load the flow cell within 10 minutes of assembly. Do not allow the flow cell to dry.

Disassemble the Slide Kit

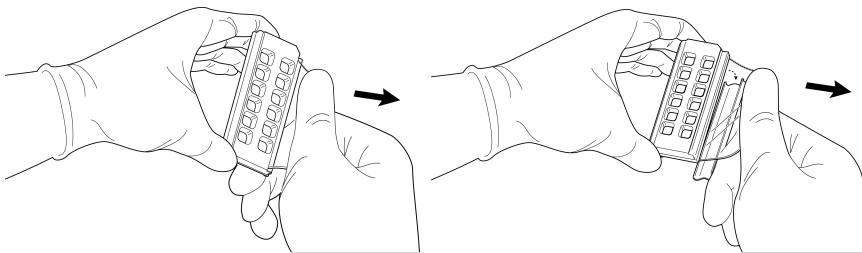
1. Wear a clean pair of gloves before disassembling the slide kit.
2. Use a vacuum aspiration system with a 200 µl tip to remove the liquid from each well of the slide kit:
 - a. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
 - b.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



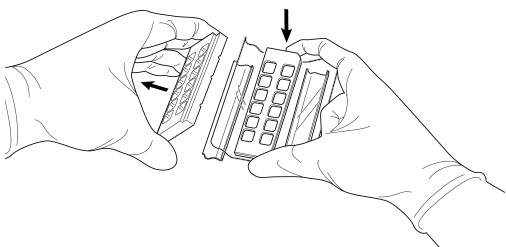
3. Hold the slide kit over the bench so clips do not fall to the ground during disassembly. Hold one side of the slide kit along the metal clip. Take care to avoid placing any pressure on the slide.
See the [video](#) on slide kit disassembly.
4. To remove the metal side clips, locate the plastic pull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



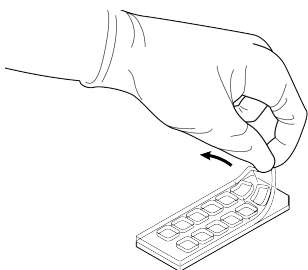
5. Grab the pull tab flap and pull the flap outwards with a firm force to dislodge the metal clip closer to the pull tab. Apply even force and avoid pulling from one spot of the pull tab.



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.



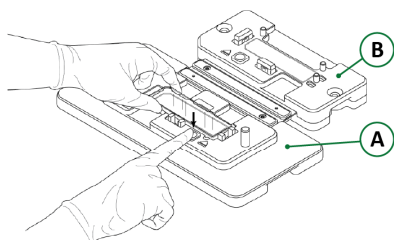
7. Lift the glass slide with the gasket and remove the plastic pull tab underneath the glass slide. Discard the pull tab.
8. Grip the top-right corner of the slide kit gasket and gently pull to remove it from the sample slide.



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

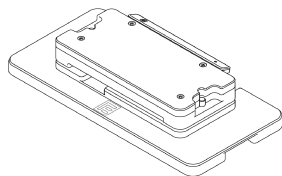
4. Open the AVITI24 Flow Cell Assembly Kit and remove the adhesive slide from the package.



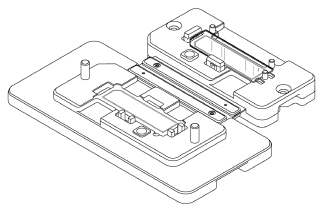
CAUTION

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.



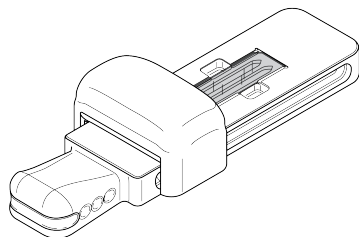
10. Clean the surface of the flow cell sealer with an isopropyl alcohol wipe. Thoroughly wipe the recessed slide holder.
For more information, see [Maintain the Flow Cell Sealer on page 50](#).



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.



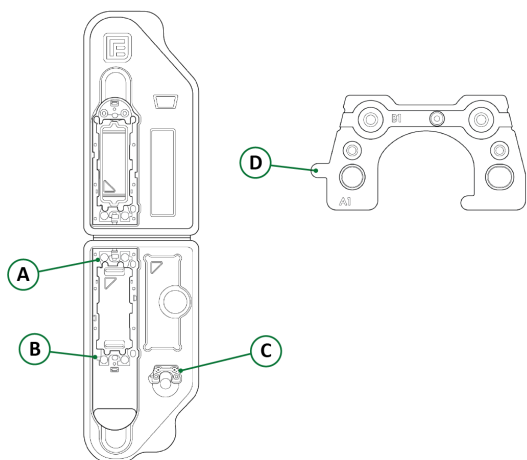
NOTE

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.



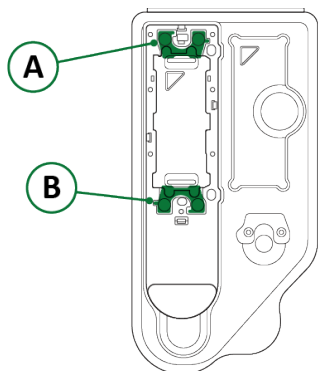
- A Gasket location above slide area
- B Gasket location below slide area
- C Gaskets stored in flow cell assembly package
- D Gasket key

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.



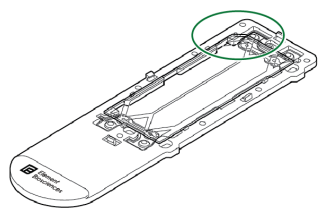
CAUTION

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

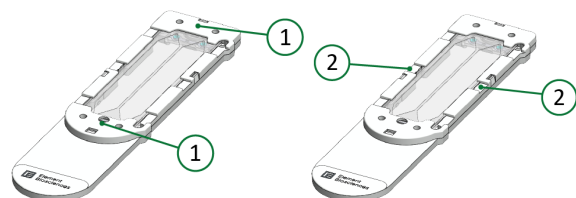


- A Gasket placed above slide area
- B Gasket placed below slide area

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



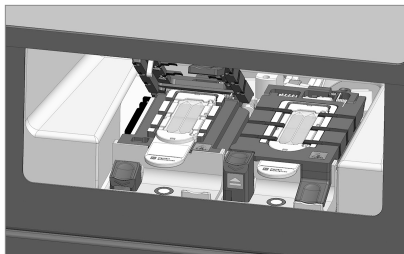
IMPORTANT

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 42](#).
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



NOTE

If you notice any errors in run set up, refer to 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 and cartridge 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 indicating 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.

The cell culture optimization run takes 7–8 hours to complete which also includes the post-run analysis time.

Monitor Run and 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—
 - » Thumbnails: Shows cell sample quality, morphology, and confluency.
 - » Cell Count, Cell Confluency, and Assigned Rate (fluorescent signal assigned to cells) are also displayed as single metrics.
3. 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.

Run Results

For a cell culture optimization run:

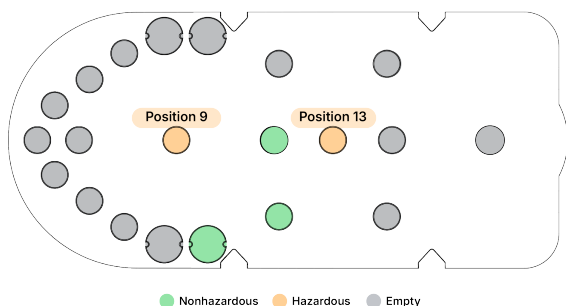
- Assess run results using CytoCanvas to inspect cell culture quality.
- Review RawCellStats.csv file for CellProfiler metrics.
- Review QC file to ensure key metrics are within the threshold values to determine your optimization run performance.

Refer to [Cell Culture Protocols for use with Cytos Cell Screening](#) for examples showing successful cell culture technique, confluency, morphology, and segmentation for some commonly used cell lines using the Cytos Onboard Optimization Kit and imaged on an AVITI24 System.

Discard the Cartridge and Bottle

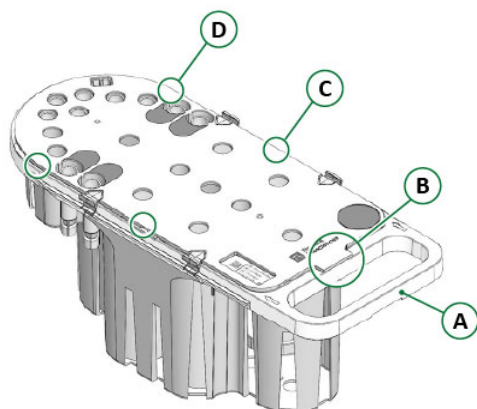
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources.

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

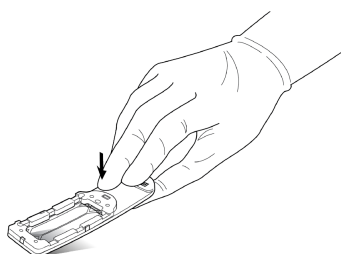
If the Flow Cell Integrity Test fails during run setup, perform the following steps to recover the flow cell.



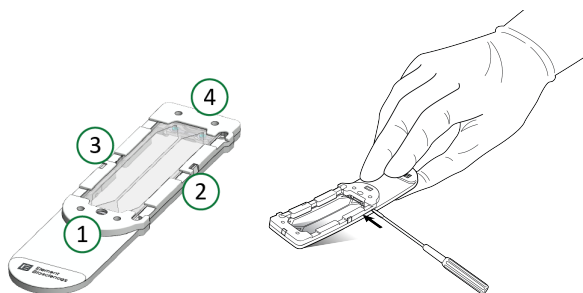
IMPORTANT

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.

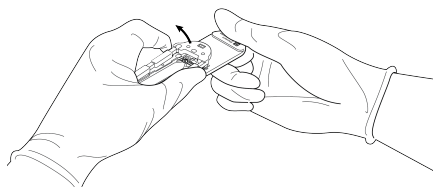
1. Open the nest and remove the flow cell.
2. Use a vacuum aspiration system or pipette at the end ports to remove all liquid from the flow cell. Make sure the flow cell is completely dry.
3. Release each of the four snap positions that secure the top half of the cassette to the bottom half in the order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

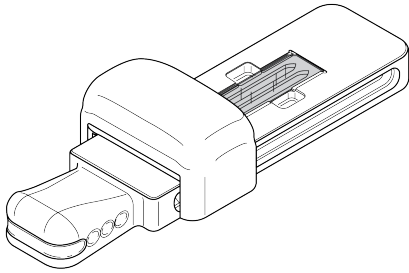


- c. With position 1 released, insert the tool at positions 2 and 3 along the side of the cassette. Gently rotate the tool to release each snap.
- d. To release the snap at position 4, rotate the top half of the cassette upward or use the tool from the side of the cassette.

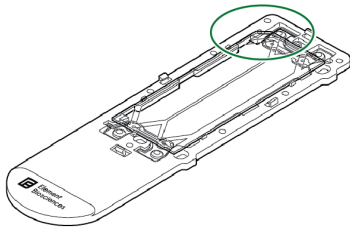


4. Lift to remove the slides from the bottom half of the flow cell cassette.
5. Re-seal the slides using the flow cell sealer:
 - a. Make sure the surface of the flow cell sealer is clean. Thoroughly wipe the flow cell sealer and the recessed slide holder.

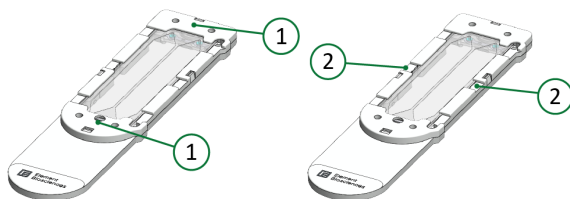
- b. Place the affixed slides in the recessed slide holder. The slides must be well-seated to avoid damage to the slides.



- 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.

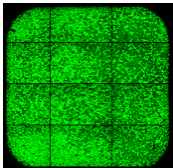
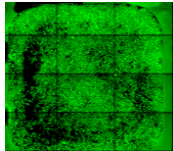
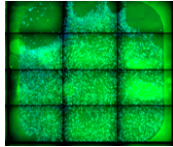
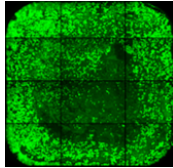
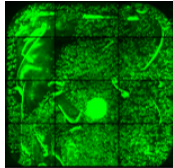
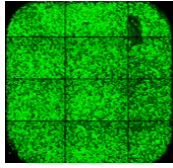
Troubleshooting

Review the information in this section to troubleshoot issues with cell culture optimization.

If a problem persists, contact Element Technical Support.

Troubleshoot Sample Preparation

The following images show results of successful and unsuccessful sample preparation on a slide kit.

Successful Result	Recommendation	
Successful preparation technique		To ensure best results, practice pipetting techniques, such as slow pipetting speed. Avoid creating bubbles or scratching the surface. Follow protocol guidelines for slide kit and pipette position as described in Protocol Guidelines on page 21 .
Unsuccessful Result	Recommendation	
Ring pattern		Avoid circular movement of the pipette or the slide kit when dispensing cells. Use a side-to-side movement for uniform cell distribution.
Cell loss in the corners of the well		Pipette slowly to prevent bubbles when dispensing cells. Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.
Cell loss in the middle of the well		Pipette slowly to reduce liquid impact on the slide surface after cell attachment. Use the half-liquid exchange method for media/reagent exchange steps by retaining a small amount of liquid in the well at each step of the fixation protocol for as described in this user guide.
Scratch marks on the surface		Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.
Cell loss in random locations		Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.

The following table provides information to address some problems during cell sample preparation and visualization.

Problem	Resolution
Cells are not properly adhered to the slide	• Review the surface coating protocol and ensure incubator settings are as recommended for cell line growth conditions. If needed, perform optimization of surface coating for your cell line using different surface coating reagents to assess and identify an optimal surface coating. • Ensure cells are healthy and exhibit > 85% viability during seeding.
Cell confluency is below desired amount after bright field microscopy	• Perform a titration to increase seeding density per well for the cell line. See Seeding Density Optimization on page 18. • Ensure cells are healthy and exhibit > 85% viability with trypan blue staining. • Verify cell count.
Cell density is higher on the edges than in the center of the well	Distribute cells by sliding the slide kit back and forth gently as described in the seeding portion of the user guide. See Culture Adherent Cells on page 22 .
Clear indicators of poor fixation such as cell loss/lifting, patches of missing cells, scratched sections of cells, or regions of low confluency/donut-ring-shaped low confluency	• Review the fixation protocols in this user guide and execute caution when fixing to prevent cells from drying out. Do not fix more than three 12-well slide kits or one 48-well or 1-well slide kit at a time for fixation. • Use the half-liquid exchange method for media/reagent exchange steps by retaining a small amount of liquid in the well at each step of the fixation protocol for 12-well or 48-well or 1-well slide kits as described in this user guide.

Troubleshoot an Optimization Run

The following troubleshooting information addresses problems that can occur during an optimization run setup using the Cytos Onboard Optimization Kit.

Problem	Resolution
Well number is shown in red indicating an error	View the thumbnails to ensure that there are cells in the well. If there are no cells, review sample preparation guidelines and fixation protocol in this guide. To ensure cells are seeded evenly across the flow cell, resuspend cells occasionally.
Uneven distribution of cells within a well	Follow the protocol guidelines in this guide to ensure proper pipetting techniques.
Majority of cells are not segmented indicating cell segmentation failure	• Review the Thumbnails panel to view cell paint images. • Perform cell culture optimization to avoid using overgrown cells that hinder proper boundary definition. Use cells at ideal confluency. • Perform resegmentation using a custom model or an appropriate model for the specific cell type. See Resegmentation Tutorial.
Nucleated rate is < 90%	Review segmentation in CytoCanvas and perform resegmentation as described above.
Missing nuclear segmentation or inaccurate borders for the nucleus	• Ensure anucleated cells were not used or cell treatment resulted in decomposition of the nucleus. • Perform cell culture optimization to avoid using overgrown cells that hinder proper boundary definition. Use cells at ideal confluency. • Perform resegmentation using a custom model or an appropriate model for the specific cell type. See Resegmentation Tutorial. • Use fresh permeabilization reagent for proper cell permeabilization.
Cell count does not match the experimental expectation	• Evaluate the experiment set up to check if any treatment was applied that inhibits or improves cell growth resulting in lower or higher cell count, respectively. • Verify cell count and ensure cells exhibit > 85% viability. • To ensure cells are seeded evenly across the flow cell, resuspend cells occasionally while seeding onto the flow cell.
Low WGA (cell membrane) intensity	• Ensure the glycosylation targets for WGA are not reduced due to any treatments or masked or the membrane is not degraded. • WGA levels are cell-line-specific. If using a cell line with low WGA, use Cytos Protein Alternative Morphology Panel with a Cytos Protein Cartridge. See Cytos Protein User Guide (MA-00095). • Use healthy, viable cells.
High WGA (cell membrane) background	WGA can bind to laminin or matrigel surface coatings 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. See Cytos Protein User Guide (MA-00095) .

Problem	Resolution
Low uniformity of phalloidin (actin) intensity	- Minimize drying of samples on the slide kit. Use half-liquid exchange method as described in this guide. - Ensure cells are distributed evenly across the well to minimize confluency inconsistency. Use the manual method or a rocker/shaker to distribute cells as described in this guide.
No CellProfiler metrics generated	Ensure the correct segmentation and analysis is selected.
Segmentation of non-cell artifacts	- Perform cell culture optimization to minimize background from cell debris, lysed proteins, or autofluorescent media components. - Perform fixation optimization to reduce cell loss.
Higher background from residual non-adhered cells, reducing cell paint image quality when low-viability cells are seeded	Verify cell count and use cells exhibiting > 85% viability for seeding.
Imaging displays bright, scattered fluorescent spots or a fluorescent smear	- Clean the flow cell and the nest as described in this guide. - Inspect nest for defects or scratches that cannot be cleaned. Do not forcefully try to remove any marks on the nest that are not easily wiped off as this can permanently damage the nest. - 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 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 Onboard Optimization Kit

Part Number and Kit Name		Quantity	Shipping	Storage
860-00056	Cytos Onboard Optimization Kit			
	Cytos Optimization Cartridge (820-00049)	1	-25°C to -15°C	-25°C to -15°C
	AVITI Buffer Bottle (820-00002)	1	Room temperature	Room temperature

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)	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

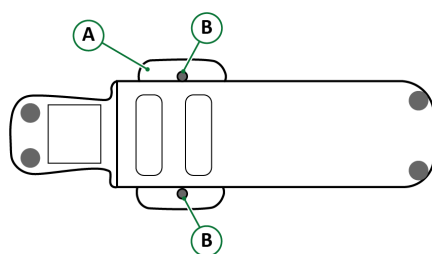
Consumable	Supplier
Isopropyl alcohol	General supplier
Ethanol (EtOH), biological grade	General lab supplier
Phosphate-buffered saline (PBS), 1X, pH 7–7.4	General lab supplier
C-Chip cell counting chamber slides	InCyto, catalog # DHC-N01
Cell culture medium appropriate for cell line	General lab supplier
Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, 1X, pH 7–7.4	ThermoFisher Scientific, catalog # 14040117
Formaldehyde (37% by weight, molecular biology)	Fisher Scientific, catalog # BP531 or similar
Microseal 'B' adhesive seals, or equivalent	Bio-Rad, catalog # MSB1001
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
μ-Plate 96 Well Square Glass Bottom	ibidi, catalog # 89627
μ-Plate 384 Well Square Glass Bottom	ibidi, catalog # 88407
Reagent trough	General lab supplier
Water bath float	General lab supplier

Consumables for Surface Coatings

Surface Coating Type	Consumable	Supplier
All surface types	0.1 N NaOH solution Biological-grade/RNase-free water	General lab supplier
Collagen coating	Collagen Type 1, 4 mg/mL, stock solution Hydrochloric acid (HCl), 0.01 N	MilliporeSigma, C3867-1VL General lab supplier
Fibronectin coating	Fibronectin stock solution	MilliporeSigma, F1141-2MG
Gelatin coating	Gelatin solution, Type B, 2% in H ₂ O	MilliporeSigma, G1393-20ML
Laminin coating	Laminin stock solution (Laminin Mouse Protein, Natural)	Gibco, 23017-015
Matrigel coating	Matrigel stock solution (Matrigel Basement Membrane Matrix)	Corning, 356237
Matrigel coating	Matrigel Growth Factor Reduced (GFR) Basement Membrane Matrix	Corning, 356231
PLL coating	PLL stock solution, 0.01%	MilliporeSigma, P4707-50ML

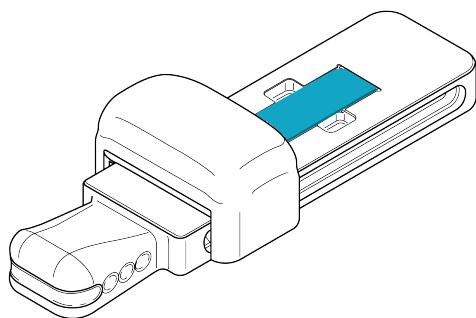
Maintain the Flow Cell Sealer

- 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.



Shipping Samples

To ship samples to another location after the fixation step, use the following instructions to prepare and package the slide kit.

1. Gather the following consumables:
 - » 1X Phosphate Buffered Saline (PBS)
 - » 40 U/μl RiboLock RNase inhibitor
 - » Adhesive seal, such as Microseal 'B'
2. If samples were stored after the fixation step, remove samples from 2°C to 8°C storage.
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. Prepare 0.1 U/μl solution of RiboLock RNase Inhibitor in 1X PBS as follows:

	48-Well or 12-Well Slide Kit	1-Well Slide Kit
RiboLock RNase Inhibitor	6 μl	15 μl
1X PBS	2.4 ml	6 ml

5. Add the appropriate volume of 1X PBS with RiboLock RNase inhibitor to each well, ensuring at least 50% of the well volume.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
50 μl	200 μl	6 ml

6. Place an adhesive seal over the wells of the slide kit. Press firmly along the edges of the wells to secure the seal.
7. Do not reuse the slide kit lid. Instead, properly dispose of the lid as waste.
8. Store samples at 2°C to 8°C until ready to ship.
9. Prepare the slide kit for shipping:
 - a. Add padding around the sealed slide kit.
 - b. Place the slide kit in an empty pipette box, small freezer box, or similar.
 - c. Place the box containing the slide kit in another box with cold packs.
 - d. Seal and label the outer box with **↑ This Side Up** to minimize impact to cells during shipping.
10. Ship samples according to local laws and regulations.

Additional Optimization Using Glass Plates

After performing initial optimization using Cytos Slide Kits, if you still need further optimization of seeding cell densities or surface coatings for your cell line, perform optimization using glass plates.

Cell culture optimization on a 96-well or 384-well glass plate with bright field microscopy allows for rapid assessment of seeding densities to quickly identify an initial compatible cell density range for a Cytos assay. Assessment can also be performed to optimize surface coating and cell culture conditions.

For seeding density titration experiments, Element recommends 96-well square, glass bottom plates (ibidi, catalog # 89627) if using a 12-well Cytos Slide Kit or 384-well square, glass bottom plates (ibidi, catalog # 88407) if using a 48-well Cytos Slide Kit for the Cytos assay.

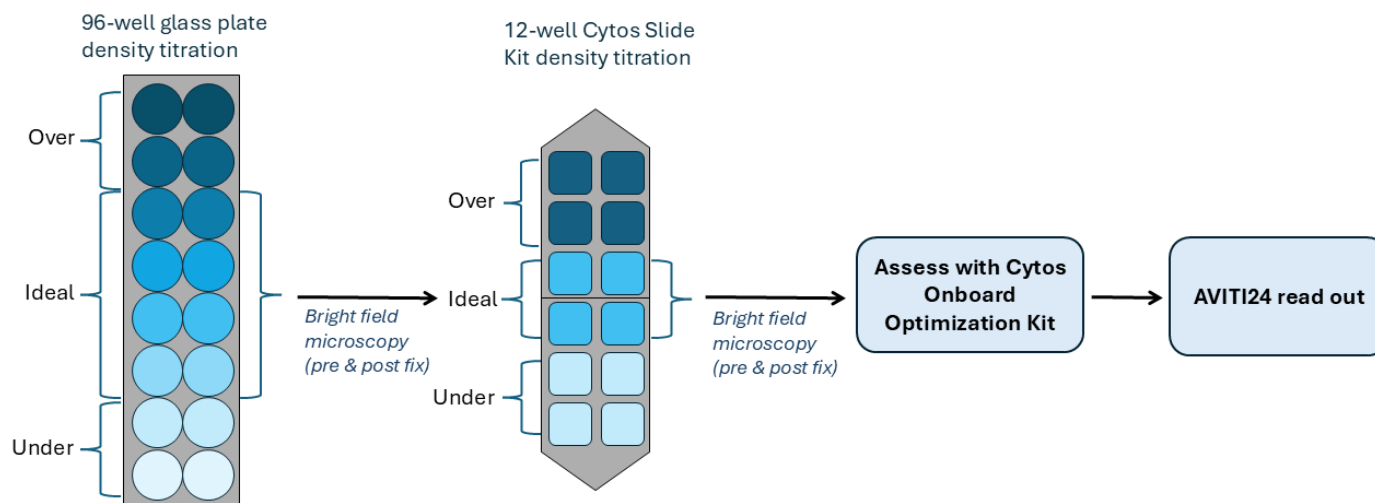
A seeding density titration is performed on the appropriate glass plate at varying cell concentrations using a logarithmic scale to allow for a wide range of cell densities. After allowing cells to grow, the cells are viewed using a bright field microscope to assess cell growth and confluency.

Guidelines and Workflow

- **Perform cell culture steps in a biosafety cabinet.**
- Perform optimization experiments in replicates and randomize the sample setup to minimize any performance bias due to well positions on the plate.
- Depending on your specific cell line, you can coat an uncoated 96-well or 384-well glass plate with commonly used surface coating reagents and assess efficacy of these different coating reagents. See [Custom Surface Coatings on page 11](#).
- Using the optimal 96-well or 384-well seeding density, perform another cell density titration on a 12-well Cytos Slide Kit or 48-well Cytos Slide Kit, respectively to identify ideal seeding density for a Cytos assay. See [Seeding Density Optimization on page 18](#)
- Use cells cultured on a glass plate at 50–70% confluency for most cells.

Seeding Density Optimization Workflow

The following workflow shows a density titration with a 96-well glass plate followed by a density titration with a 12-well Slide Kit. The cells were imaged before and after sample fixation using bright field microscopy. The cells at ideal confluency are then assessed with Cytos Onboard Optimization Kit and imaged on an AVITI24 System. A similar density titration can also be performed with a 384-well glass plate and 48-well Cytos Slide Kit.



Culture and Fix Adherent Cells

1. Gather the following consumables:
 - » Cell culture medium appropriate for the cell line
 - » Healthy cells growing at 60–80% confluency in a tissue culture flask
 - » 96-well square, glass bottom plate (ibidi, catalog # 89627) or 384-well square, glass bottom plates (ibidi, catalog # 88407) coated with a custom surface coating for your cell type
2. Warm the cell culture medium in a 37°C water bath.
3. If you prepared a custom surface coating, remove any liquid stored in the wells of the glass plate.
4. Wash each well with the appropriate volume of prewarmed culture medium. Slightly tip the plate and slowly dispense along the wall of each well. Do not contact the plate surface. Ensure the media covers the surface in each well.

384-Well Glass Plate	96-Well Glass Plate
50 µl	200 µl

5. To remove liquid, slightly tip the plate and position the pipette tip in the corner of the well. Do not contact the plate surface.
6. Repeat wash one more time.
7. Ensure cells are fully dissociated to single cells and counted. Resuspend the cells to a target concentration. Element uses HeLa cells resuspended at **270–300 K cells per ml**.
8. Holding the pipette perpendicular to the well, gently load the appropriate volume of suspended cells in the center of each well. Do not swirl the pipette when loading. Do not contact the plate surface.

384-Well Glass Plate	96-Well Glass Plate
40 µl	150 µl

Use the following example of HeLa cells as a guide for seeding density range set up on a glass plate. Be sure to set up the experiment in replicates.

Well	384-Well Glass Plate (Cells/Well)	96-Well Glass Plate (Cells/Well)
A	10,667	40,000
B	8000	30,000
C	5333	20,000
D	2667	10,000
E	2000	7500
F	1067	4000
G	533	2000
H	133	500

9. Gently distribute the cells for 30 seconds using a forward-and-back, pause for 5 seconds, and then side-to-side motion.
10. Incubate the cells at **37°C for 16 to 18 hours** or as appropriate for your cell line to target ideal confluency in each well.
11. View cells using bright field microscopy to ensure cells are well adhered and healthy. Capture representative bright field microscopy images pre-fixation. See [Assess Cell Sample Using Bright Field Microscopy on page 20](#).
12. Gather the following consumables for fixing cells:
 - » Warm 1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, pH 7–7.4, sterilized (ThermoFisher Scientific, catalog # 14040117)
 - » Warm 1X Phosphate Buffered Saline (PBS), pH 7–7.4

» Freshly prepared Formaldehyde, 4% (Fixation reagent), dilute as needed with DPBS

13. To remove liquid, slightly tip the plate and position the pipette tip in the corner of the well. Do not contact the plate surface.

14. Wash each well with the appropriate volume of 1X DPBS. Slightly tip the slide kit and slowly dispense along the well wall.

384-Well Glass Plate	96-Well Glass Plate
50 µl	200 µl

15. To remove liquid, slightly tip the plate and position the pipette tip in the corner of the well. Do not contact the plate surface.

16. Repeat wash **two more times**.

17. Slowly, add the appropriate volume of fixation reagent along the wall of each well. Cover the plate with a lid.

384-Well Glass Plate	96-Well Glass Plate
40 µl	150 µl

18. Incubate at **room temperature for 20–30 minutes**.

19. To remove the fixation reagent, slightly tip the plate and position the pipette tip in the corner of the well. Do not contact the plate surface. Remove all liquid in the well before proceeding to the wash steps.

20. Wash cells with the appropriate volume of 1X PBS.

384-Well Glass Plate	96-Well Glass Plate
50 µl	200 µl

21. Repeat the wash **two more times**. Do not remove the liquid after the final wash.

22. View cells using bright field microscopy to identify a target seeding density to obtain ideal confluency for your cell type. Capture representative bright field microscopy images post-fixation. See [Assess Cell Sample Using Bright Field Microscopy on page 20](#).

Culture and Fix Suspension Cells

- Gather the following consumables:
 - » 1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, pH 7–7.4
 - » Healthy cells growing at 60–80% confluency in a tissue culture flask
 - » 96-well square, glass bottom plate (ibidi, catalog # 89627) or 384-well square, glass bottom plates (ibidi, catalog # 88407) coated with a custom surface coating for your cell type
 - » 15 ml or 50 ml Falcon tube
- Centrifuge the cells for 5 minutes at 300 x g in a 15 ml or 50 ml Falcon tube depending on final volume.
- Remove the supernatant without disturbing the cell pellet.
- Add 1X DPBS to resuspend the cell pellet in an appropriate volume to a target concentration. Element uses Jurkat cells resuspended at **3.5–4 million cells per ml**.
- Holding the pipette perpendicular to the well, gently load the appropriate volume of suspended cells in the center of each well. Do not swirl the pipette when loading. Do not contact the plate surface.

384-Well Glass Plate	96-Well Glass Plate
40 µl	150 µl

Use the following example of Jurkat cells as a guide for seeding density range set up on a glass plate. Be sure to set up the experiment in replicates.

Well	384-Well Glass Plate (Cells/Well)	96-Well Glass Plate (Cells/Well)
A	133,333	500,000
B	80,000	300,000
C	64,000	240,000
D	48,000	180,000
E	32,000	120,000
F	16,000	60,000
G	8000	30,000
H	2667	10,000

- Cover the plate with a lid and centrifuge the plate at **300 x g for 15 minutes**.
- Remove the plate assembly from the centrifuge and remove the plate from the holder.
- View cells using bright field microscopy. Capture representative bright field microscopy images pre-fixation. See [Assess Cell Sample Using Bright Field Microscopy on page 20](#).
- Gather the following consumables for fixing cells:
 - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4, sterilized
 - » Freshly prepared Formaldehyde, 8% (Fixation reagent), dilute as needed with PBS
- Remove the lid from the plate. Do not remove any liquid from the wells.
- Slowly, add the appropriate volume of fixation reagent along the wall of each well. **Do not pipette to mix**. Cover the plate with a lid.

384-Well Glass Plate	96-Well Glass Plate
40 µl	150 µl

- Incubate at **room temperature for 20–30 minutes**.

13. To remove the fixation reagent, slightly tip the plate and position the pipette tip in the corner of the well. Do not contact the plate surface. Remove all liquid in the well before proceeding to the wash steps.

14. Wash cells with the appropriate volume of 1X PBS.

384-Well Glass Plate	96-Well Glass Plate
50 µl	200 µl

15. Repeat the wash **two more times**. Do not remove the liquid after the final wash.

16. View cells using bright field microscopy to identify a target seeding density to obtain ideal confluency for your cell type. Capture representative bright field microscopy images post-fixation. See [Assess Cell Sample Using Bright Field Microscopy on page 20](#).

Document History

Revision	Description of Change
October 2026 Document # MA-00094 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



Element Biosciences
10055 Barnes Canyon Road, Suite 100
San Diego, CA 92121
elementbiosciences.com
Telephone: 619.353.0300
Email: info@elembio.com

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