Element Biosciences

Teton™ CytoProfiling

User Guide

FOR USE WITH

AVITI24™ System
AVITI Operating Software v3.4 or later
Teton Cartridge and Teton Fixed Panels
Teton Custom Add-On Protein Panels
Teton Focus Protein Panels
Teton Atlas™ Cartridge, Low Output

ELEMENT BIOSCIENCES
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Document # MA-00053 Rev. F
December 2025

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CHAPTER 1

Overview

A cytoprofiling run on the AVITI24 System performs Avidite Base Chemistry™ (ABC™) sequencing within cell samples to detect numerous cellular RNA and proteins. The workflow requires prepared cell samples and Teton consumables.

This guide provides instructions for preparing samples, assembling the flow cell, and performing a cytoprofiling run. Before initiating a run, make sure you have read the instrument overview and safety information in the *AVITI24 System User Guide (MA-00051)*.

Cytoprofiling 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 cytoprofiling run. The run is complete when the recipe is executed and primary analysis is complete.

- **Cell Paint**—Uses a combination of reversible dyes and organelle probes coupled to oligo barcodes. Avidites bind to barcodes and the fluorescence intensity is imaged to detect cellular features.
- **Amplification**—Oligo barcodes are amplified using rolling circle amplification (RCA) to form polonies.
- **Batches**—Polonies are sequenced over multiple batches using a batch-specific sequencing primer that binds to the target.

Cytoprofiling Run Consumables

A cytoprofiling run on the AVITI24 System requires a reagent cartridge and buffer bottle, a slide kit, and flow cell assembly kit. Additional kits are required depending on which reagent cartridge you use and how you customize your run. To perform a dual flow cell run, a quantity of two of each kit is required.

Two types of reagent cartridges are available for runs on an AVITI24 System: the Teton cartridge and the Teton Atlas cartridge.

Teton Cartridge

The Teton cartridge provides reagents for a Teton run with the exception of protein and RNA targets, which are provided in Teton fixed panel kits. Each Teton fixed panel kits provides a protein tube with 50 protein targets and an RNA tube with 350 RNA targets. After thawing the Teton reagent cartridge, fixed panel tubes are loaded onto the Teton cartridge before the run. See *Teton Fixed Panels* on page 6.

You can customize the run with an optional custom protein panel. See *Teton Full Custom Protein Panel* on page 6 and *Teton Focus Protein Panels* on page 6.

Teton Atlas Cartridge, Low Output

The Teton Atlas cartridge, low output, provides reagents for a Teton Atlas low-output run. The cartridge includes removable tubes for a custom protein panel and two required custom primers.

Custom primers for the Teton Atlas cartridge are designed in ElemBio Cloud Custom Designer™. See the *ElemBio Cloud Online Help* and the *Teton Atlas Custom Primer Design Technical Note (LT-00060)*. After designing the primers, you can order from a third-party vendor. Custom primers are added to the Teton Atlas cartridge before the run. See *Teton Atlas Low Output Protocol* on page 25.

For custom protein panel options, see *Teton Full Custom Protein Panel* on page 6 and *Teton Focus Protein Panels* on page 6.

Teton Fixed Panels

Required with the Teton cartridge, each Teton fixed panel includes a 350-plex RNA panel and a 50-plex protein panel. The RNA and protein panels are provided in tubes that are designed to load directly onto the Teton cartridge. See *Teton Fixed Panel Kits* on page 44 for available fixed panels for the Teton cartridge.

For a list of targets associated with each fixed panel, see the following documentation:

- *Teton Human MAPK RNA and Protein Targets (MA-00062)*
- *Teton Human Neuro Panel RNA and Protein Targets (MA-00071)*
- *Teton Human Immuno Panel RNA and Protein Targets (MA-00072)*

Teton Full Custom Protein Panel

A full custom protein panel using the Teton Custom Add-On Protein Panel Assembly Kit enables up to 88 additional protein targets of your choosing for a run on the AVITI24 System. Designing a custom protein panel requires access to the ElemBio Cloud Custom Designer. See *Teton Full Custom Protein Panel* on page 19.

To assess the selected protein targets in the custom add-on panel, use the Teton Custom Antibody Screening Kit to prepare for a short imaging run on the AVITI24 System. Results from the run help identify targeted antibodies for the custom protein panel. For more information, see the *Teton Optimization & Screening User Guide (MA-00078)*.

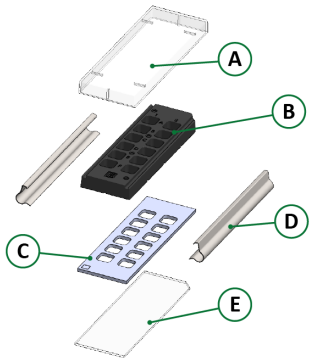
Teton Focus Protein Panels

Each of the nine available Teton Focus Protein Panels include a selection of 24 barcoded protein targets. To avoid barcode conflicts, panels are grouped in three sets with one possible selection from each set. Optionally, you can use up to two panels and add up to a 24-plex Teton Custom Add-On Protein Panel. Designing a custom protein panel requires access to the ElemBio Cloud Custom Designer. See *Teton Focus Protein Panels* on page 22.

Within each set is an option for immunology, cell biology, or neuroscience. For a list of protein targets associated with each focus panel, see *Teton Focus Protein Panel Targets (MA-00077)*.

Teton Slide Kit

The Teton 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, and a lid. The frame and the gasket determine the number of wells on the slide. Before starting a Teton run, the slide is reassembled as a flow cell with parts from the Teton Flow Cell Assembly Kit.

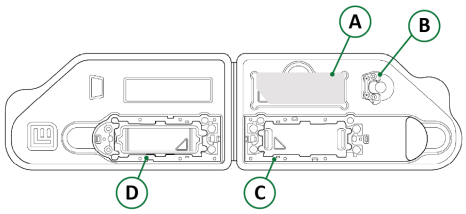


A Lid
B Frame
C Gasket
D Side clip
E Glass slide

Slide kits are available in 48-well, 12-well, or 1-well configurations, and for each configuration either PLL-coated or uncoated surfaces. Uncoated slide kits provide the option of applying a custom surface specific to your cell line. See *Custom Surface Coatings* on page 8.

Teton Flow Cell Assembly Kit

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

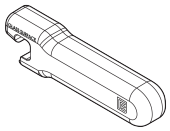


A Adhesive slide
B Flow cell gaskets (2)
C Frame bottom
D Frame top

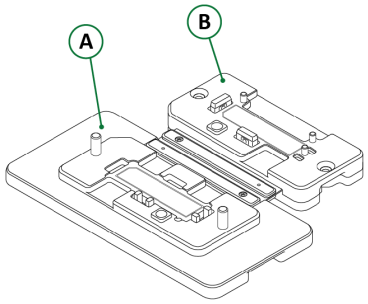
Teton Flow Cell Assembly Tools

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

- **Teton Slide Kit Tool**—The Teton slide kit tool assists in disassembling the slide kit. See *Disassemble the Slide Kit* on page 34.

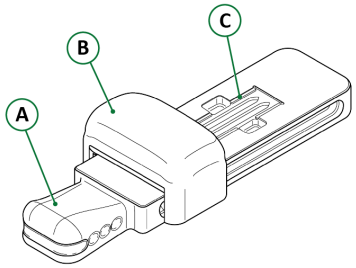


- **Teton Flow Cell Aligner**—The Teton flow cell aligner aligns and adheres the sample slide from the slide kit to the adhesive slide provided in the Teton Flow Cell Assembly Kit. See *Align and Seal the Slides* on page 36.



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

- **Teton Flow Cell Sealer**—The Teton 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 *Caring for the Teton Flow Cell Sealer* on page 47.



A Base grip
B Roller grip
C Indentation for slides

CHAPTER 2

Custom Surface Coatings

Uncoated Teton 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. If you are using a PLL-coated slide kit, a custom surface is not necessary.

Perform all steps in a biosafety cabinet.

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
   - » Teton Slide Kit
2. 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 46.
3. **For the 48-well slide kit only**—Gently tap the slide kit a few times on both sides and visually inspect for bubbles.
   - » Make sure that no bubbles are trapped in the bottom of the wells.
   - » If bubbles are present, centrifuge the slide kit at 130 x g for 2.5 minutes. Balance the slide kit in the centrifuge.
4. Incubate at room temperature for 15 minutes inside the biosafety cabinet.
5. Remove the solution from each well.
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 5 more times. Remove the final wash solution from each well.
8. Proceed to the preparation steps for your preferred surface coating:
   - » *Collagen Coating* on page 9
   - » *Fibronectin Coating* on page 9
   - » *Gelatin Coating* on page 10
   - » *Laminin Coating* on page 10
   - » *Matrigel Coating* on page 11
   - » *Poly-L-Lysine (PLL) Coating* on page 12

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 H_2O—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
25 µl	80 µl	1 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
25 µl	80 µl	1 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 µl	150 µl	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 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.

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 0.5–1 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)
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4, chilled
3. Thaw matrigel at 2°C to 8°C for 1 hour or until it liquifies and appears less viscous.
   - » Avoid multiple thaw cycles. Store small quantities of matrigel at -25°C to -15°C for up to 2 years.
   - » When thawed, swirl the vial of matrigel to ensure all the material is dispersed.
4. If not already chilled, chill the 1X PBS at 2°C to 8°C for 30 minutes.
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
25 µl	80 µl	1 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.

Poly-L-Lysine (PLL) Coating

Teton slide kits are available with a PLL-coating. For a user-applied PLL coating on an uncoated slide kit, use the following instructions.

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 7 days.

CHAPTER 3

Sample Preparation

Sample preparation on a Teton slide kit is required for all Teton runs on an AVITI24 System. This section describes sample preparation for both adherent cells and suspension cells.

- *Culture and Fix Adherent Cells* on page 14
- *Attach and Fix Suspension Cells* on page 16

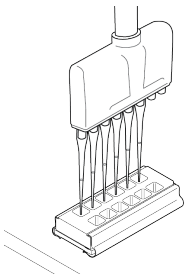
NOTE
Cells expressing fluorescent proteins can interfere with Teton readouts and are not recommended.

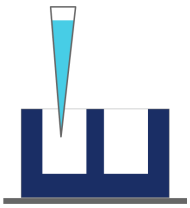
Optimize seeding densities and cell culture conditions for your specific cell line using 96-well or 384-well glass plates and bright field microscopy. For more information, see the *Teton Optimization & Screening User Guide (MA-00078)*.

To determine the success of sample preparation, use the Teton Cell Paint Probe Kit (part # 830-00035) to view samples on a fluorescent microscope, or use the Teton Onboard Cell Paint Imaging Kit (part # 860-00047) to view samples after a short run on the AVITI24 System. For more information, see the *Teton Optimization & Screening User Guide (MA-00078)*.

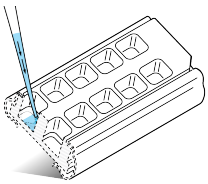
Protocol Guidelines

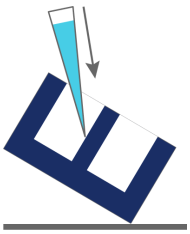
- **Perform sample preparation steps in a biosafety cabinet.** Steps involving live cells ***must*** be performed in a biosafety cabinet.
- Avoid disturbing the slide surface throughout the protocol.
- Do not allow cells to dry out. Allowing cells to dry out can result in cell detachment.
- Ensure proper pipette placement during on-flow cell treatments.



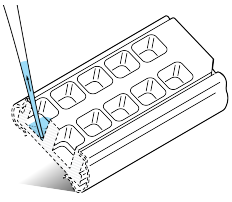


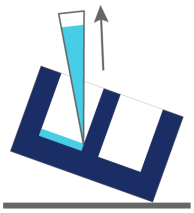
- When dispensing cells, hold the pipette perpendicular to the glass slide surface and dispense into the center of each well.
- Do not contact the slide surface.
- Do not swirl the pipette when loading.
- To reduce performance variability, ensure the slide kit is on a flat and level surface during cell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





- When removing liquid, position the pipette tip in the corner of the well.
- Do not contact the slide surface.

Culture and Fix Adherent Cells

Sample preparation of adherent cells involves steps to culture cells on the Teton 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.

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

Culture Adherent Cells

1. Gather the following consumables:
   - » Cell culture medium appropriate for the cell line
   - » Teton Slide Kit
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.
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 Finnpipette. See *User-Supplied Consumables* on page 46.

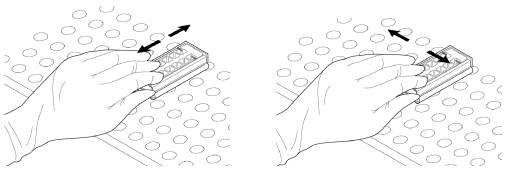
5. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
6. Repeat the wash one more time.
7. Ensure the cells are fully dissociated to single cells and counted.
8. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently load 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 following information to estimate initial cell 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. Gently distribute the cells for 30 seconds using a forward-and-back, 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.



10. **For the 48-well slide kit only**—Place the slide kit in a slide kit tray (part # 860-00044) 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 30–70% in each well. Do not allow cell overgrowth.

    As an example, Element incubates HeLa cells for 16 to 18 hours.

Fix Cultured Adherent Cells

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
   - Formaldehyde, 4% (Fixation reagent), dilute as needed with DPBS
   - If storing the slide, 40 U/µl RiboLock RNase inhibitor diluted to 0.1 U/µl with 1X PBS
2. Warm the 1X DPBS in a 37°C water bath.
3. To remove the cell culture medium, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
4. Carefully wash each well with the appropriate volume of 1X DPBS to remove dead cells. Slightly tip the slide kit and slowly dispense along the wall of each well.

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

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. Slightly tip the slide kit and slowly add the appropriate volume of fixation reagent along the wall of each well.

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

8. With a lid on the slide kit, incubate at room temperature for 20–30 minutes.

   Fixation time varies by cell line. Do not exceed 30 minutes.
9. 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.
10. Carefully wash each well with the appropriate volume of *1X PBS*. Slightly tip the slide kit and slowly add liquid along the wall of each well.

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

11. 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.
12. Repeat the wash two more times. Do not remove the liquid after the final wash.
13. After fixing cells, proceed to one of the following options:
    - Customize a protein panel. See *Teton Full Custom Protein Panel* on page 19 or *Teton Focus Protein Panels* on page 22.
    - Perform a cytoprofiling run on the AVITI24 System. See *Run Preparation and Setup* on page 27.
    - If you plan to store the samples, see *Prepare Cell Samples for Storage* on page 18.
    - If you plan to ship samples, see *Shipping Samples* on page 48.

Attach and Fix Suspension Cells

Sample preparation of suspension cells involves steps to attach cells to the Teton 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.

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

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
   - » Teton Slide Kit
   - » 15 ml or 50 ml Falcon tube
2. To ensure DPBS remains sterile, always open the DPBS bottle inside the biosafety cabinet.
3. Centrifuge the cells for 5 minutes at 300 x g in a 15 ml or 50 ml Falcon tube depending on final volume.
4. Remove the supernatant without disturbing the cell pellet.
5. Add 1X DPBS to resuspend the cell pellet and dilute the cell solution depending on desired confluency.
6. With the slide kit on a flat and level surface, hold the pipette perpendicular to the well and gently load 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.

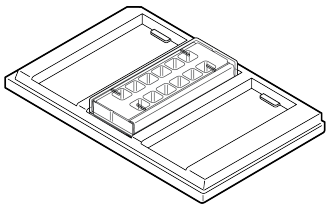
48-Well Slide Kit	12-Well Slide Kit
40 µl	150 µl

NOTE
For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpipette. See *User-Supplied Consumables* on page 46.

Use the following information to estimate initial cell seeding density. Optimize depending on cell size or final application.

	48-Well Slide Kit	12-Well Slide Kit
Well size:	3.5 mm x 3.5 mm	7 mm x 7 mm
Element uses:	16–24 K Jurkat cells per well	60–90 K Jurkat cells per well

7. Cover the wells with the slide kit lid.
8. Load the covered slide kit onto a Teton 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.



9. Centrifuge at 300 x g for 15 minutes.

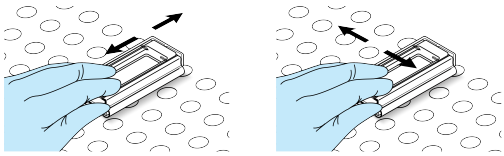
Attach Suspension Cells on a 1-Well Slide Kit

1. Gather the following consumables:
   - » 1X Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, pH 7–7.4
   - » Teton Slide Kit
   - » 15 ml Falcon tube
2. To ensure DPBS remains sterile, always open the DPBS bottle inside the biosafety cabinet.
3. Centrifuge the cells for 5 minutes at 300 x g in a 15 ml or 50 ml Falcon tube depending on final volume.
4. Remove the supernatant without disturbing the cell pellet.
5. Add 5 ml 1X DPBS to resuspend the cell pellet and dilute the cell solution depending on desired confluency.
6. 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.

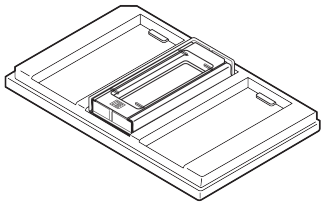
   Use the following information to estimate initial cell seeding density. Optimize depending on cell size or final application.

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

7. Cover the wells with the slide kit lid.
8. Gently distribute the cells for 30 seconds using a forward-and-back, 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 well.



9. Incubate at room temperature for 15 minutes.
10. Load the covered slide kit onto a slide kit tray (part # 860-00044).
    - » Load only one slide in the center position.
    - » Balance the centrifuge with another slide kit tray.



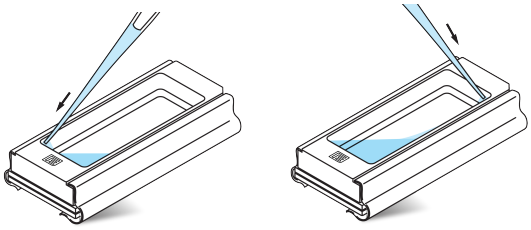
11. Centrifuge at 20 x g for 2 minutes.

Fix Attached Suspension Cells

1. Gather the following consumables:
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
   - » Formaldehyde, 8% (Fixation reagent), dilute as needed with DPBS
   - » If storing the slide, 40 U/µl RiboLock RNase inhibitor diluted to 0.1 U/µl with 1X PBS
2. Remove the assembly holder from the centrifuge, remove the slide kit from the holder, 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.

   Fixation time varies by cell line. 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
50 µl	200 µl	3 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. After fixing cells, proceed to one of the following options:
    - » Customize a protein panel. See *Teton Full Custom Protein Panel* on page 19 or *Teton Focus Protein Panels* on page 22.
    - » Perform a cytoprofiling run on the AVITI24 System. See *Run Preparation and Setup* on page 27.
    - » If you plan to store the samples, see *Prepare Cell Samples for Storage* on page 18.
    - » If you plan to ship samples, see *Shipping Samples* on page 48.

Prepare Cell Samples for Storage

1. Remove liquid from the final wash.
2. 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
25 µl	60 µl	1 ml

3. Cover the wells with an adhesive seal and store samples at 2°C to 8°C for up to 30 days.

CHAPTER 4

Teton Full Custom Protein Panel

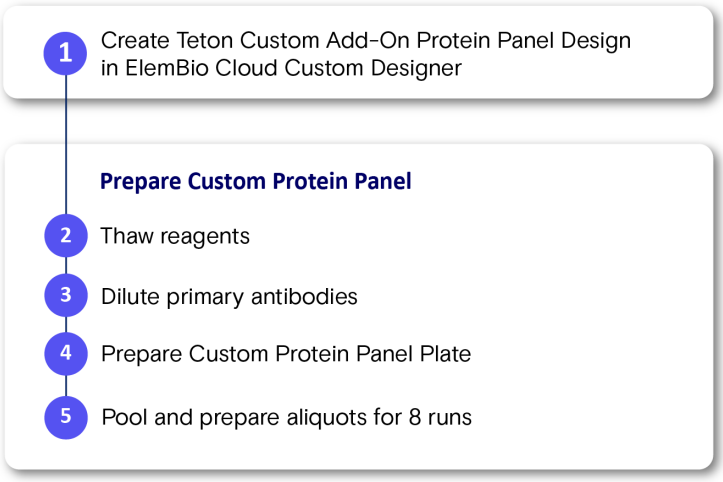
A full custom protein panel using the Teton Custom Add-On Protein Panel Assembly Kit enables an additional 88 protein targets of your choosing to customize a cytoprofiling run with targets of your interest. Your selection of up to 88 targets are pooled with the protein tube for the run. This add-on option is compatible with the Teton cartridge (part # 820-00036) and the Teton Atlas cartridge (part # 820-00039).

The Teton Custom Add-On Protein Panel Assembly Kit includes a reagent plate with different Teton detection probes in each well to combine with your selected protein targets. Custom protein panels of 24-plex or fewer require a Teton Diversity Spike-In for Teton cartridge. See *Teton Custom Add-On Protein Panel Kits* on page 44.

To design your custom protein panel, use the interactive ElemBio Cloud Custom Designer. Your custom protein panel can be attached to a planned run or imported for a manual run from the cloud or USB drive as a panel.json file.

To ensure the best selections for a custom protein panel, you can first use the Teton Custom Antibody Screening Kit and perform a custom antibody screening run on the AVITI24 System. Include the down-selected antibodies in your custom protein design. For more information, see the *Teton Optimization & Screening User Guide (MA-00078)*.

Protocol Summary



Prerequisites and Planning

1. Confirm that your primary antibodies are compatible.

   NOTE
   Only rabbit antibodies are compatible with this protocol.

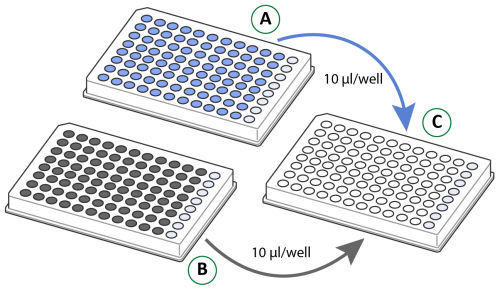
2. Go to the **ElemBio Cloud Custom Designer**.
3. Follow the onscreen instructions to create a new custom protein panel design and plate layout.

Thaw Reagents and Dilute Primary Antibodies

1. Remove the following kit components from -25°C to -15°C storage:
   - » Teton Custom Add-On Protein Buffer
   - » Teton Custom Add-On Protein Control
   - » Teton Custom Plate (do not remove from pouch)
2. Thaw the Teton Custom Add-On Protein Buffer in a room temperature water bath for 15 minutes. Set aside on ice.
3. Thaw the Teton Custom Add-On Protein Control and Teton Custom Plate at room temperature for 15 minutes. Set aside on ice.
4. Use one of the following methods to dilute each primary antibody to 3.4 µg/ml (22 nM) with Custom Add-On Protein Buffer:
   - » Dilute directly in each well of your 96-well primary antibody plate.
   - » Dilute in tubes and then transfer the diluted primary antibodies to a 96-well plate.

Prepare the Custom Protein Panel Plate

1. When thawed, remove the Teton Custom Plate from the pouch and briefly centrifuge.
2. Remove the foil seal and pipette to mix each well.
3. Label a new 96-well plate **custom protein panel plate**.
4. Transfer 10 µl from wells A1–H1 of the Teton Custom Plate to the corresponding well of the custom protein panel plate using a multichannel pipette.



A Teton Custom Plate (Teton Custom Add-On Protein Panel Kit)
B Diluted primary antibodies (3.4 µg/ml)
C Custom protein panel plate (combined wells)

5. Transfer 10 µl from each well of your diluted primary antibody plate to the corresponding well of the custom protein panel plate. Pipette to mix after each transfer.
6. Seal the custom protein panel plate and briefly centrifuge to remove bubbles.
7. Incubate at room temperature for 1 hour.

Pool and Aliquot a Full Custom Protein Panel

1. Add 18 µl from each well of the custom protein panel plate to a 2 ml tube.

   For an 88-plex plate, expect a pooled volume of 1584 µl.
2. If your custom panel is fewer than 88-plex, add the appropriate volume of Teton Custom Add-On Protein Buffer to the pooled panel to result in a volume of 1620 µl using the following formula:

   1584 – (Plexity x 18) = Buffer volume

   For example, if your panel is 72-plex, add 288 µl Teton Custom Add-On Protein Buffer.
3. Add 36 µl of the Teton Custom Add-On Protein Control to the pooled panel.

   For an 88-plex plate, expect a pooled volume of 1620 µl.

4. Using eight low-bind tubes, transfer 200 µl pooled protein panel to each tube.
5. Label each tube with panel plexity and sample information.
6. Store unused aliquots at -25°C to -15°C for up to 30 days.
7. To add a full custom protein panel to a run, see *Run Preparation and Setup* on page 27.

CHAPTER 5

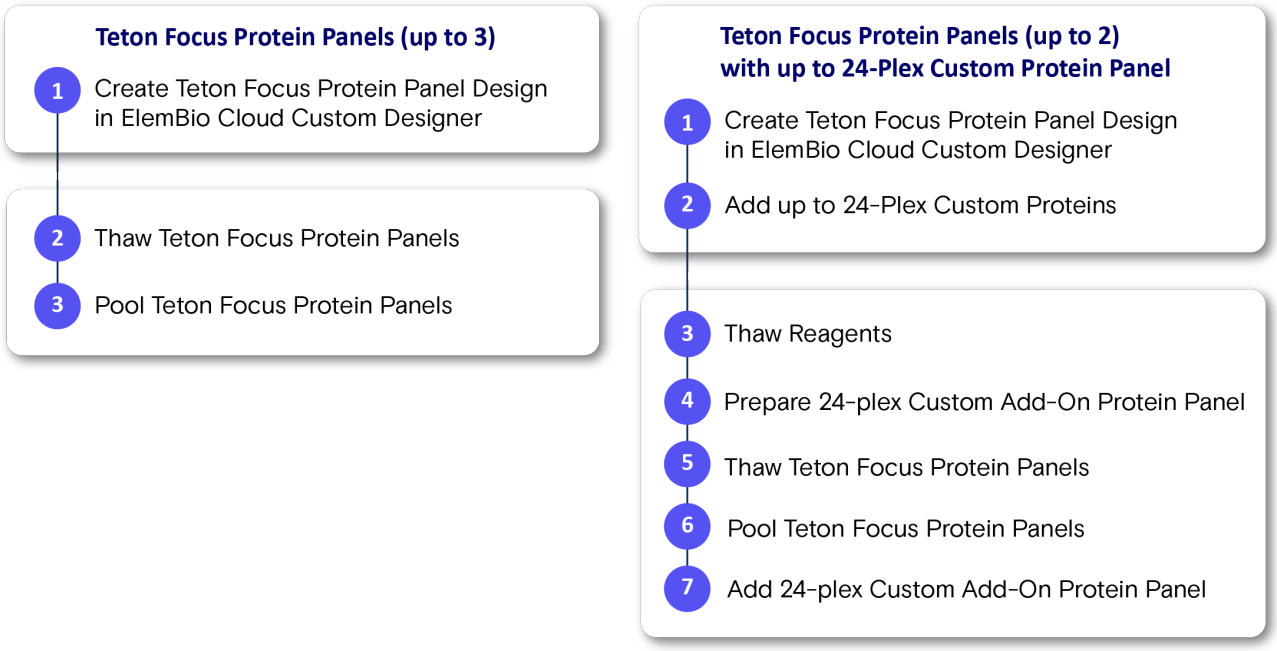
Teton Focus Protein Panels

Teton Focus Protein Panels are 24-plex fixed protein panels that you can combine for a cytoprofiling run. All nine panels are grouped in three sets based on barcode compatibility. To avoid conflicting barcodes, select only one focus panel from each set. Use the ElemBio Cloud Custom Designer to select compatible panels and design your run.

	Set 1	Set 2	Set 3
Immunology	Cytokine Signaling (830-00046)	T Cell Activation (830-00047)	Innate Immunity (830-00048)
Cell Biology	Cell Metabolism (830-00049)	Cell Stress & Apoptosis (830-00050)	Gene Regulation (830-00051)
Neuroscience	Neurodegeneration (830-00052)	Neurodevelopment (830-00053)	Neuroinflammation (830-00054)

You can design your run with up to three Teton Focus Protein Panels or a combination of up to two Teton Focus Protein Panels and a 24-plex Teton Custom Add-On Protein Panel.

Protocol Summary



Create a Teton Focus Protein Panel Design

1. Go to the **ElemBio Cloud Custom Designer**.
2. Follow the onscreen instructions to select compatible focus panels.
3. [Optional] Add a 24-plex Teton Custom Add-On Protein Panel.
4. Order Teton Focus Protein Panels and Teton Custom Add-On Protein Buffer. For ordering information, see *Teton Focus Protein Panels* on page 45.

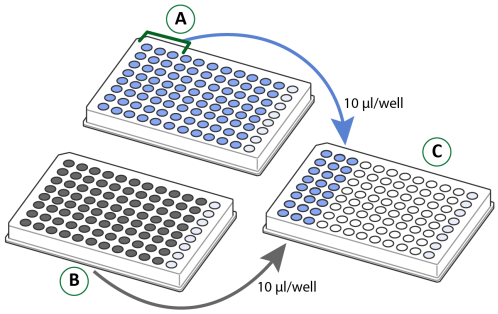
Thaw Reagents for Custom Add-On Panel

1. Remove the following components from -25°C to -15°C storage:
   - » Teton Custom Add-On Protein Buffer
   - » Teton Custom Plate (do not remove from pouch)
2. Thaw the Teton Custom Add-On Protein Buffer in a room temperature water bath for 15 minutes. Set aside on ice.
3. Thaw the Teton Custom Plate at room temperature for 15 minutes. Set aside on ice.

Prepare a 24-Plex Custom Protein Panel Plate

1. When thawed, remove the Teton Custom Plate from the pouch and briefly centrifuge.
2. Remove the foil seal and pipette to mix each well.
3. Label a new 96-well plate **custom protein panel plate**.
4. Transfer 10 µl from wells A1–H3 of the Teton Custom Plate to the corresponding well of the custom protein panel plate using a multichannel pipette.

   NOTE
   You must use wells A1–H3 to avoid barcode conflict with the Teton Focus Protein Panels.



A Teton Custom Plate (Teton Custom Add-On Protein Panel Kit)
B Diluted primary antibodies (3.4 µg/ml)
C Custom protein panel plate (combined wells A1–H3 only)

5. Transfer 10 µl from each well of your diluted primary antibody plate to the corresponding well of the custom protein panel plate. Pipette to mix after each transfer.
6. Seal the custom protein panel plate and briefly centrifuge to remove bubbles.
7. Incubate at room temperature for 1 hour.

Pool and Aliquot a 24-Plex Protein Panel

1. Add 18 µl from each well of the custom protein panel plate to a 2 ml tube.
   - » For a 24-plex plate, expect a pooled volume of 432 µl, which supports 8 reactions.
   - » *Do not* add Teton Custom Add-On Protein Control to the 24-plex pooled panel.
2. If your custom panel is fewer than 24-plex, add the appropriate volume of Teton Custom Add-On Protein Buffer to the pooled panel to result in a volume of 432 µl using the following formula:

   432 – (Plexity x 18) = Buffer volume

   For example, if your panel is 16-plex, add 144 µl Teton Custom Add-On Protein Buffer.
3. Using eight low-bind tubes, transfer 52 µl pooled protein panel to each tube.
4. Label each tube with panel plexity and sample information.
5. Store unused aliquots at -25°C to -15°C for up to 30 days.

Thaw Teton Focus Protein Panels

1. Time the thawing of Teton Focus Protein Panels with run preparation steps. See *Thaw Reagent Cartridge* on page 28.
2. Remove the Teton Focus Protein Panels from -25°C to -15°C storage.
3. Thaw each Teton Focus Protein Panel on ice for 20 minutes. Invert the tube several times to mix.
4. If not already thawed, remove the Teton Custom Add-On Protein Buffer from -25°C to -15°C storage, and thaw the Teton Custom Add-On Protein Buffer in a room temperature water bath for 15 minutes. Set aside on ice.

Pool Teton Focus Protein Panels

1. Add 76 µl from each Teton Focus Protein Panel to a 2 ml tube.
2. If combining a 24-plex custom add-on protein panel:
   - » Add 48 µl pooled custom add-on protein panel to the pooled Teton Focus Protein Panels. Pipette to mix.
   - » *Do not* add Teton Diversity Spike-In.
3. Add the appropriate volume of Teton Custom Add-On Protein Buffer for a total volume of 228 µl.

Protein Panels	Protein Volume	Buffer Volume
3 Focus Protein Panels (76 µl each)	228 µl	0 µl
2 Focus Protein Panels (76 µl each) + 24-plex Custom Add-On Protein Panel (48 µl)*	200 µl	28 µl
2 Focus Protein Panels (76 µl each)	152 µl	76 µl
1 Focus Protein Panel (76 µl) + 24-plex Custom Add-On Protein Panel (48 µl)*	124 µl	104 µl
1 Focus Protein Panel (76 µl)	76 µl	152 µl

* Up to a 24-plex Teton Custom Add-On Protein Panel can be combined with up to two Teton Focus Protein Panels.

4. Gently pipette to mix and then briefly centrifuge. *Do not vortex.*
5. Proceed to *Run Preparation and Setup* on page 27.

CHAPTER 6

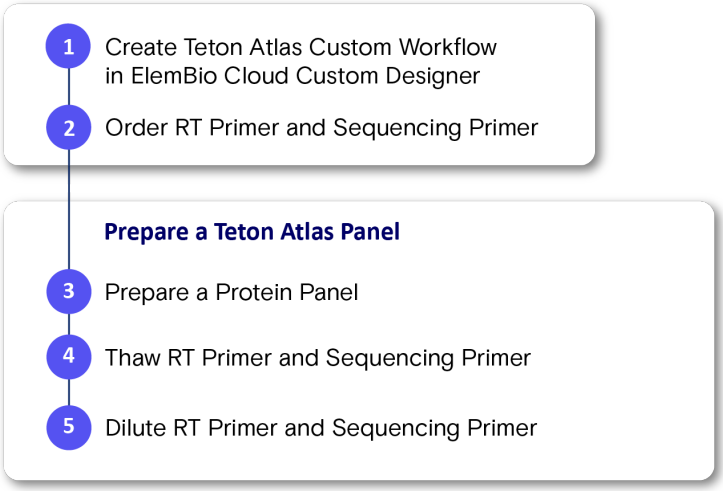
Teton Atlas Low Output Protocol

The Teton Atlas low-output protocol enables a cytoprofiling run with 3-plex cell paint and up to 100 bp Direct In Sample Sequencing. Additionally, include an 88-plex custom protein panel or a pool of Teton Focus Protein Panels in the Teton Atlas assay. See *Teton Full Custom Protein Panel* on page 19 and *Teton Focus Protein Panels* on page 22.

The Teton Atlas low-output kit includes a Teton Atlas cartridge, a buffer bottle, and a Teton Reagent Kit with cell paint reagents. One kit supports one run on the AVITI24 System. If performing a run on both side A and side B, two kits are required. Additionally, the Teton Atlas protocol requires the Teton Atlas RNA Spike-In, which supports up to eight runs.

A Teton Atlas run requires two custom primers per target designed using ElemBio Cloud Custom Designer: the reverse transcription (RT) primer and the sequencing primer. After the compatible oligo sequences are generated, order the primers from a third-party oligo vendor. Your custom design can be attached to a planned run or imported for a manual run from the cloud or USB drive. For more information, see the *ElemBio Cloud Custom Designer online help* and the *Teton Atlas Custom Primer Design Technical Note (LT-00060)*.

Protocol Summary



Design the Teton Atlas Custom Workflow

1. Go to the **ElemBio Cloud Custom Designer**.
2. Follow the onscreen instructions to design the panel:
   a. For the Teton Atlas low-output cartridge, 3-plex cell paint is selected by default.
   b. Select an option for the protein panel, either a full custom add-on protein panel or selection of Teton Focus Protein Panels.
   c. Define sequencing target sites to generate compatible oligo sequences for the RT primer and sequencing primer.
   d. If applicable, create a Target Cell Assignment manifest.

   NOTE
   ElemBio Cloud Custom Designer provides the RT primer and sequencing primer that is compatible to your target site.
3. Order the RT primer and sequencing primer with the specified sequences from a third-party oligo vendor.

Prepare a Custom Protein Panel

Prepare a custom protein panel using one of the following custom protein options:

- **Full Custom Protein Panel**—Prepare up to an 88-plex Teton Full Custom Protein Panel. See *Teton Full Custom Protein Panel* on page 19.
- **Teton Focus Protein Panels with optional Custom Add-On Protein Panel**—Select up to three Teton Focus Protein Panels or up to two Teton Focus Protein Panels with an additional 24-plex Teton Custom Add-On Protein Panel. See *Teton Focus Protein Panels* on page 22.

Thaw Reagents and Custom Primers

1. Time the thawing of custom primers with run preparation steps. See *Thaw Reagent Cartridge* on page 28.
2. When the reagent cartridge is almost thawed, remove the custom RT primer, custom sequencing primer, and Teton Atlas RNA Spike-In from -25°C to -15°C storage.
3. Thaw the RT primer and sequencing primer at room temperature for 15 minutes. Gently invert each tube several times to mix. Set aside on ice.
4. Thaw the Teton Atlas RNA Spike-In at room temperature for 15 minutes. Set aside on ice.

Dilute Custom Primers

1. Dilute the custom sequencing primer to 100 µM using nuclease-free water. Set aside on ice.
2. Dilute the custom RT primer to 100 µM using nuclease-free water. Set aside on ice.
3. Further dilute the RT primer using a serial dilution 1:100 to result in 1 µM. Set aside on ice.
4. Proceed to *Run Preparation and Setup* on page 27.

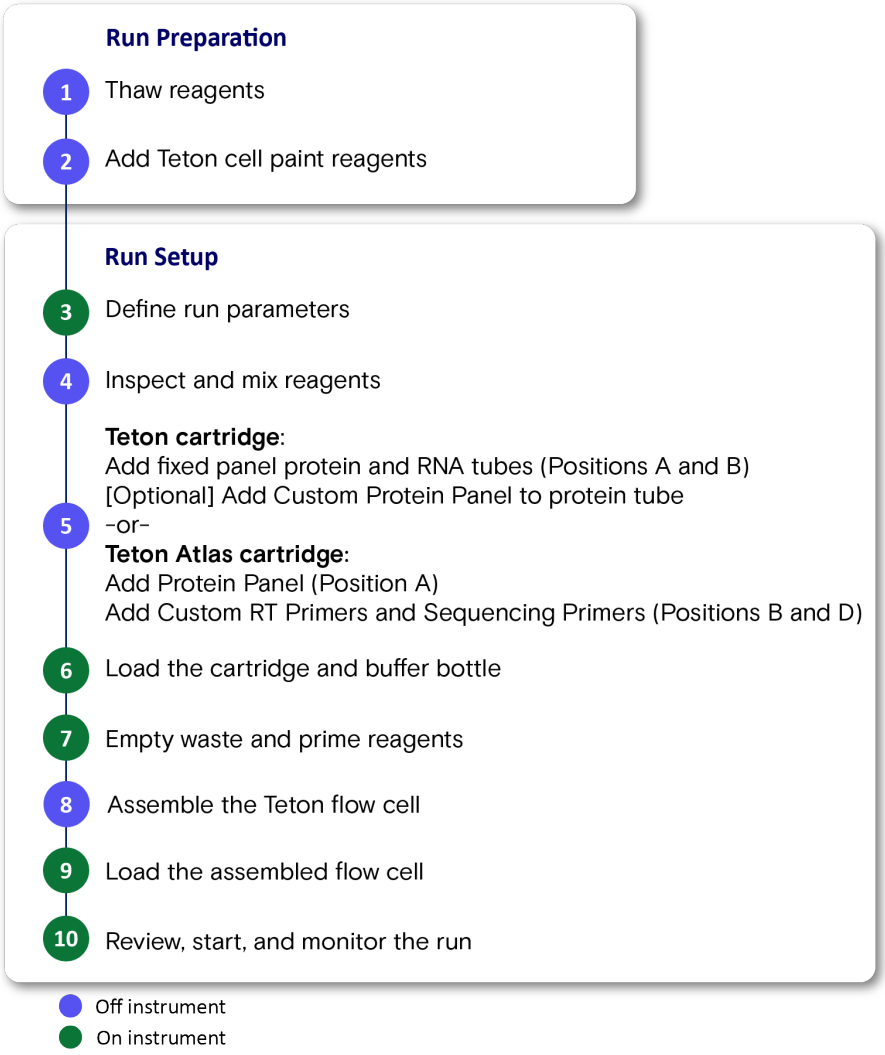
CHAPTER 7

Run Preparation and Setup

Performing a cytoprofiling run on an AVITI24 System includes steps to add cell paint reagents, assemble the flow cell, and then follow prompts on the AVITI OS interface to setup the run.

Cell samples must be prepared on a Teton slide kit in advance of run preparation and setup. See *Sample Preparation* on page 13. Additionally, any custom protein panels and primers must be prepared in advance and added to the thawed reagent cartridge. See *Teton Full Custom Protein Panel* on page 19, *Teton Focus Protein Panels* on page 22, or *Teton Atlas Low Output Protocol* on page 25.

Workflow Summary



Prepare Reagents

Preparing reagents for a run requires thawing the reagent cartridge and cell paint reagents. For a Teton cartridge, thaw Teton Fixed Panel tubes.

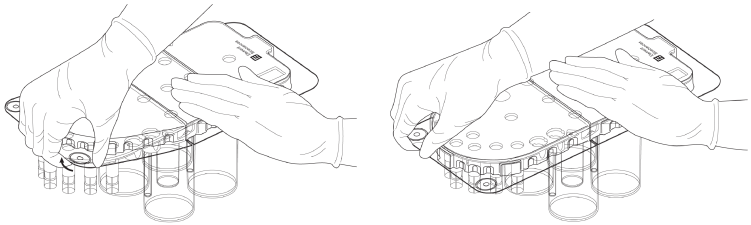
Thaw Reagent Cartridge

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.
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. Set aside the thawed cartridge at room temperature. If not immediately initiating the run, place the thawed cartridge at 2°C to 8°C. Do not exceed 3 hours.

Thaw Cell Paint Reagents

1. When the reagent cartridge is almost thawed, remove Teton Reagent A and Teton Reagent B from -25°C to -15°C storage.

   NOTE
   Cell paint reagents are provided in the Teton Reagent Kit (part # 830-00055).
2. Thaw reagents in a room temperature water bath for 20 minutes.

Thaw Fixed Panel Tubes for a Teton Cartridge

1. When the reagent cartridge is almost thawed, remove the protein tube and RNA tube provided in the fixed panel kit from -25°C to -15°C storage.
2. Thaw reagents in a room temperature water bath for 15 minutes.

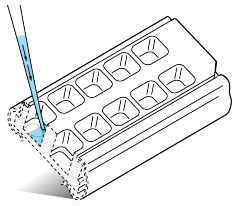
Add Cell Paint Reagents

1. Ensure Teton Reagent A and Teton Reagent B are thawed.
2. Invert each tube 10 times to mix and then briefly centrifuge. *Do not vortex*.
3. Remove prepared samples on slide kit from 2°C to 8°C storage.
4. To remove the liquid from the slide kit, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
5. Wash each well with the appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of each well wall. Do not pipette up and down.

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

6. 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.
7. Slightly tip the slide kit and slowly add the appropriate volume of Teton Reagent A along the middle of each well wall.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
25 µl	80 µl	1.5 ml

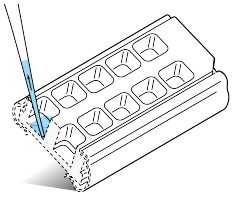


NOTE
Fill volumes for Teton Reagent A and Teton Reagent B provided in the Teton Reagent Kit (part # 830-00055) enable the use of a trough and multichannel pipette with 48-well and 12-well slide kits.

8. Incubate at room temperature for 10 minutes.
9. 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.
10. Wash each well with the appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of the wall of each well.

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

11. 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.



12. Repeat the wash two more times.
13. Slightly tip the slide kit and slowly add the appropriate volume of Teton Reagent B along the middle of the wall of each well.

48-Well Slide Kit	12-Well Slide Kit	1-Well Slide Kit
25 µl	80 µl	1.5 ml

14. Incubate at room temperature for 10 minutes.
15. 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.
16. Wash each well with the appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of the wall of each well.

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

17. 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.
18. Repeat the wash two more times. Do not remove the liquid after the final wash.
19. Leave the cells in 1X PBS. Proceed to *Set Up a Cytoprofiling Run*.

Set Up a Cytoprofiling Run

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

   NOTE
   For Teton Atlas runs that include Target Cell Assignment, two manifests are required. One is the run manifest, which provides cell type information for segmentation. The second is the Target Cell Assignment Manifest, which assigns target regions to cells.
2. If you are using a Teton Cartridge with Teton Add-On Protein Panel designed in ElemBio Cloud Custom Designer, download the custom protein panel as a panel.json file which is uploaded during the run set up to allow for custom protein batch sequencing.
3. On the Home screen, select **New Run**.
4. For run type, select **Cytoprofiling**.
5. Select a side or both sides to use for the run.
   - » **Side A**—Set up a run on side A.
   - » **Both**—Set up simultaneous runs on sides A and B.
   - » **Side B**—Set up a run on side B.
6. Select either **Teton** or **Teton Atlas** based on the cartridge you are using.
7. Select **Next** and proceed to one of the following steps:
   - » For a **Manual Run**, proceed to *Define Manual Run Parameters*.
   - » For a **Planned Run**, proceed to *Select a Planned Run*.

Define Manual Run Parameters

1. Make sure **Manual Run** is selected for the type of run.
2. In the Run Name field, enter a unique name to identify the run.

   The field accepts 1–64 alphanumeric characters, hyphens (-), and underscores (_).
3. [Optional] In the Run Manifest field, select **Browse** and import a run manifest.

   You can import a run manifest from an inserted USB drive or from an SMB storage connection.
4. [Optional] In the Description field, enter a description that represents the run.

   The field accepts ≤ 500 alphanumeric characters, hyphens, underscores, spaces, and periods (.).
5. In the Storage drop-down menu, select a storage location or leave the default selection.
6. In the Well Layout field, select **48**, **12**, or **1** well.

7. If using a **Teton cartridge**:
   a. Select the fixed panel you are using from the **Fixed Panel** drop-down menu.
   b. For runs with a Teton Add-On Protein Panel designed in ElemBio Cloud Custom Designer, import the panel as follows:
      - For instruments with access to ElemBio Cloud, select the name of your panel from the drop-down menu.
      - Otherwise, select **Upload a panel.json file**, navigate to an inserted USB drive or storage location, and select the file.
8. If using a **Teton Atlas cartridge**:
   a. Select the Teton Atlas custom workflow for the run as follows:
      - For instruments with access to ElemBio Cloud, select the name from the DISS Workflow drop-down menu.
      - Otherwise, select **Upload a Workflow Definition Package**, navigate to an inserted USB drive or storage location, and select the file.
   b. For runs with Target Cell Assignment included, select the **Target Cell Assignment manifest** from the TCA Manifest drop-down menu.
9. For the Small Cell option, select **No**. This is a rarely used feature as it reduces polony density within cells. Contact Element Biosciences for guidance on cell lines to use.
10. For the Expanded Z option, select **No**. This is a rarely used feature. May be suitable for cells that exhibit strong cell-cell adhesion and grow in 3-dimensional clusters. Contact Element Biosciences for guidance on cell lines to use.
11. If you are using a custom recipe, select **Advanced Settings**. Select **Browse** and import the custom recipe file from a USB drive.
12. Select **Next** and repeat steps 2–12 to setup side B, if applicable.
13. Proceed to *Inspect and Mix Reagents* on page 31.

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 for Cytoprofiling* in the *Online Help*.
2. Select the run you want to use from the list of planned runs.
3. Review the run parameter fields to make sure they are correct.

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

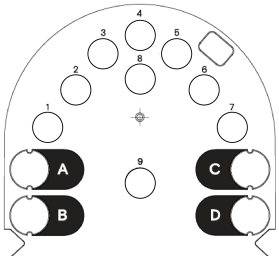
Inspect and Mix Reagents

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

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

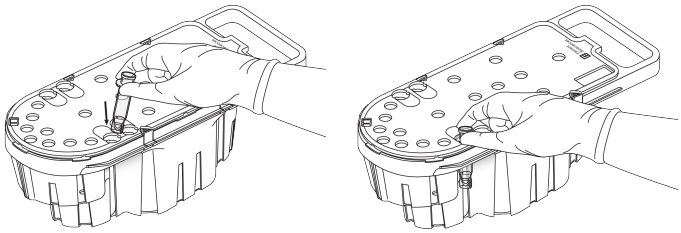
Add Fixed Panel Tubes to the Teton Cartridge

A Teton fixed panel protein tube and RNA tube are required for a Teton cartridge in positions A and B.

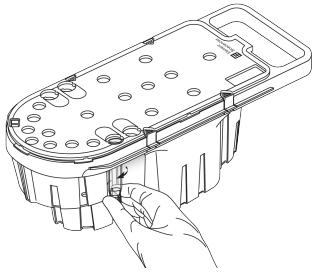


Position **A**—Protein tube
Position **B**—RNA tube

1. Gently invert the thawed Teton fixed panel protein tube to mix.
2. From the top of the cartridge, insert the protein tube in position **A**.



3. Hold the bottom of the tube and turn clockwise 90° to lock the tube in position. Push upward to confirm a locked position.



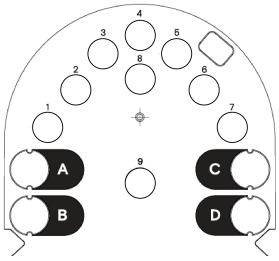
4. Gently invert the Teton fixed panel RNA tube to mix.
5. From the top of the cartridge, insert the RNA tube in position **B**.
6. Hold the bottom of the tube and turn clockwise 90° to lock the tube in position. Push upward to confirm a locked position.

Add Custom Protein Panel to the Protein Tube

1. Using a clean pipette tip, pierce the foil seal of the protein tube in position **A** of the cartridge.
2. **If you are using a Full Custom Protein Panel**:
   a. Add 180 µl pooled protein panel to the protein tube of the **Teton** or **Teton Atlas cartridge**. Pipette to mix.
   b. For a **Teton cartridge**—If your custom panel is 24-plex or fewer, add 11 µl Teton Diversity Spike-In to the protein tube. Pipette to mix.
3. **For Teton Focus Protein Panels**—Add the full volume of pooled Teton Focus Protein Panels (228 µl) to the protein tube of the **Teton** or **Teton Atlas cartridge**. Pipette to mix.

Add Custom Primers to the Teton Atlas Cartridge

Teton Atlas custom RT primer and sequencing primer are required for a Teton Atlas cartridge in positions B and D.



Position **A**—Protein tube
Position **B**—RNA RT Primer tube
Position **D**—RNA Sequencing Primer tube

1. Using a clean pipette tip, pierce the foil seal of the RNA RT Primer tube in position **B** of the cartridge.
2. Add 25 µl of each custom RT primer to the RNA RT Primer tube. Gently pipette to mix.
3. Add 3 µl Teton Atlas RNA Spike-In to the RNA RT Primer tube. Gently pipette to mix.
   Use the RT primer pool on the same day.
4. Using a clean pipette tip, pierce the foil seal of the RNA Seq Primer tube in position **D** of the cartridge.
5. Add 20 µl of each custom sequencing primer to the RNA Seq Primer tube. Gently pipette to mix.

Confirm Reagent Preparation

The checkboxes that appear on the AVITI OS screen depend on which customization protocols are used for the run.

1. Select **Invert cartridge** to confirm that reagents are mixed.
2. Select **Insert into basket** to confirm that the cartridge is in the cartridge basket.
3. **For a Teton cartridge**
   - » Make sure the fixed panel protein tube is present on the cartridge:
     - ◦ If using a fixed panel only, select **Invert and load protein tube**.
     - ◦ If using a custom protein panel with a fixed panel, select **Verify add-on protein and load protein tube**.
   - » Make sure the RNA fixed panel tube is present on the cartridge, and select **Invert and load RNA tube**.
4. **For a Teton Atlas cartridge**
   - » Make sure the protein panel is present on the cartridge, and select **Verify add-on protein and load protein tube**.
   - » Make sure the RT primers are present on the cartridge, and select **Verify RNA RT primers and load RNA tube**.
   - » Make sure the sequencing primers are present on the cartridge, and select **Verify RNA seq primers and load seq primer tube**.
5. Make sure the flow cell assembly is complete with no gaps in the sides of the flow cell, and select **Verify flow cell**.
6. 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, you can discard run setup and save the cartridge. Priming pierces reagent seals and prevents further use.
8. Select **Next** to *automatically* start priming. Priming takes approximately 24 minutes.
9. When priming is complete, proceed to *Assemble the Teton Flow Cell*.

Assemble the Teton Flow Cell

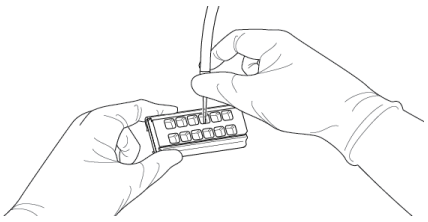
To assemble the Teton flow cell, gather the following tools, kits, and recommended equipment:

- Vacuum aspiration system with 200 µl tip (recommended)
- Teton Slide Kit Tool
- Teton Flow Cell Aligner and Teton Flow Cell Sealer
- Teton Flow Cell Assembly Kit

> For best results, complete the flow cell assembly within 10 minutes and load the flow cell immediately after assembly.

Disassemble the Slide Kit

1. 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.



Perform step **2** to remove the metal side clips using the Teton slide kit tool (part # 810-00021). Otherwise, perform step **3**.

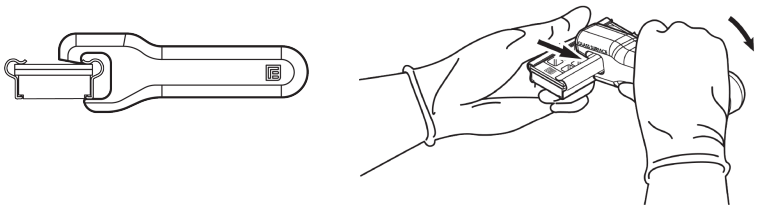
2. Remove metal side clips *using the Teton slide kit tool*:

   a. Hold one side of the slide kit along the metal clip. Take care to avoid placing any pressure on the slide.

   b. On the other side, *gently* place the Teton slide kit tool in the center of the slide kit along the metal clip. Make sure the hooked shape of the tool is positioned under the lip of the metal clip.

   CAUTION
   Avoid damage to the slide. Position the slide kit tool along the center of the metal side clips. Hold the slide kit along the edges to avoid applying pressure to the slide surface.

   c. Holding the side of the slide kit firmly with your hand, rotate the tool handle downward to release the clip.



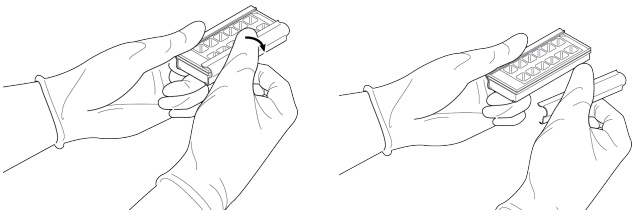
   d. Repeat step **2** to remove the metal side clip from the opposite side of the slide kit.

3. Remove metal side clips *using your fingers:*

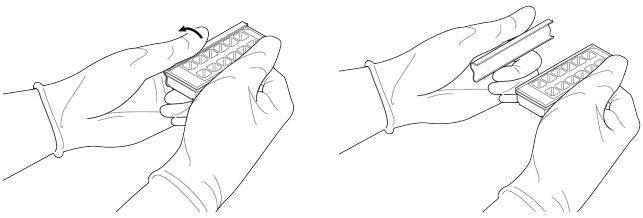
   a. Turn the slide kit upside down so the open wells are facing downward and the glass slide is facing upward.

   b. Holding the slide kit with both hands on the long edges, place your thumb on the top-center location of one of the side clips. With smooth and consistent movement, rotate the top edge of the side clip outward to release the clip.

   CAUTION
   Avoid damage to the slide. Do not pull the side clip from the end regions of the clip. Always pull the clips from the center of each clip.

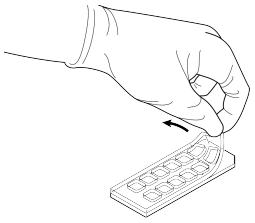


   c. To release the second clip, place your thumb on the top-center location of the side clip, and rotate the top edge of the side clip outward with smooth and consistent movement.



4. 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.

5. Grip the top-right corner of the slide kit gasket and gently pull to remove it from the sample slide.



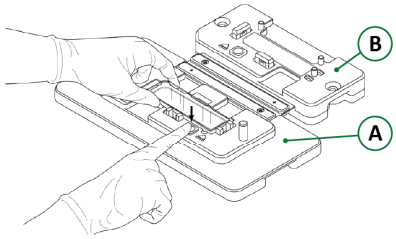
Align and Seal the Slides

1. Make sure the surface of the Teton flow cell aligner is clean. Thoroughly wipe both sides of the aligner, 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. Load the sample slide onto the Teton 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.



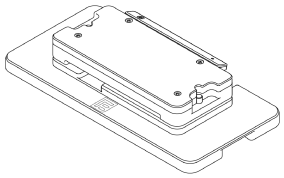
A **Sample** side
B **Adhesive** side

3. Open the Teton Flow Cell Assembly Kit and remove the adhesive slide from the package. Handle the slide from the edges only and make sure the slide is free of debris.
4. 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.

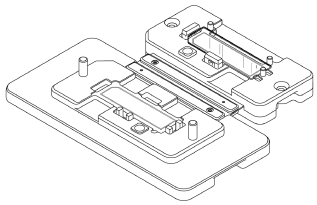
5. Starting from the beveled corner, peel off the protective easy-peel film from the adhesive slide.
6. Close the aligner to affix the sample slide and adhesive slide:

   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.

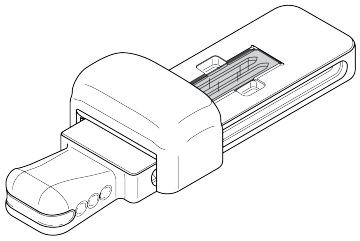


7. Gently place your hand on the aligner for 5 seconds. Excessive pressure can damage the slides.

8. With both hands, lift the **Adhesive** side to open the flow cell aligner. The sample slide is affixed to the adhesive slide.



9. Clean the surface of the Teton flow cell sealer with an ethanol wipe. Thoroughly wipe the recessed slide holder.
   For more information, see *Caring for the Teton Flow Cell Sealer* on page 47.
10. 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.
11. Place the aligned slides on the flow cell sealer in the recessed slide holder. Make sure the slides are well-seated.



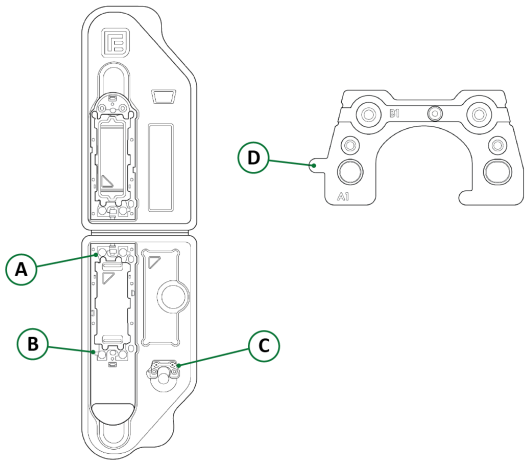
12. Hold the flow cell sealer roller grip with one hand and the base handle with the other hand. *Slowly* move the roller grip forward and then back, taking ~2 seconds to roll in each direction. With each roll, make sure the slides remain well-seated in the recessed area. Repeat the forward and back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

    NOTE
    Moving the roller grip slowly ensures a proper seal and avoids damage to the slides.
13. Flip over the aligned slides and place in the recessed slide area of the flow cell sealer. Repeat step 12 an additional 3 times.

Assemble the Flow Cell Cassette

1. 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.

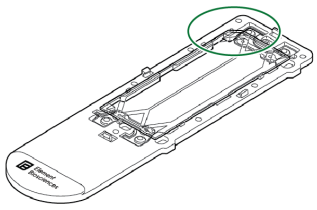
   CAUTION
   Gaskets ***must*** be present to prevent run failure.



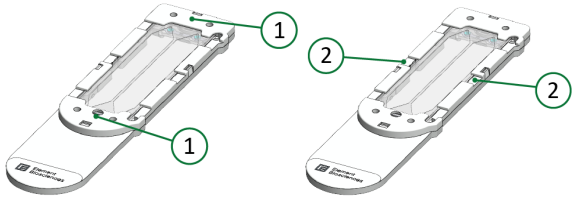
A Gasket location above slide area
B Gasket location below slide area
C Gaskets stored in flow cell assembly package
D Gasket key

2. Remove the slides from the flow cell sealer.

3. 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.



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



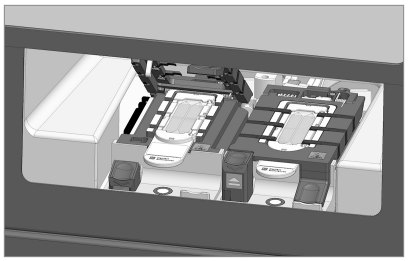
6. Visually inspect the flow cell cassette to make sure there are no gaps along the sides. If a gap is visible, repeat step **5** to ensure the top and bottom frames are fully engaged.

Clean the Assembled Flow Cell

1. Wipe the assembled flow cell surface with an ethanol wipe.
2. Dry the surface with a lens wipe.
3. Use canned air to ensure the flow cell is free of dust.
4. Proceed immediately to *Load the Flow Cell*.

Load the Flow Cell

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. With the label facing up, place the assembled Teton flow cell over the three registration pins on the nest.



6. Lower the tab on the right side of the lid until the lid snaps into place.
   —The nest status light turns green.—
7. Select **Close Nest** to close the nest bay door and retract the stage.
8. 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.
9. 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
Panel	The fixed panel for the run (applicable to a Teton cartridge only)
Storage	The location where run output is stored
Manifest	The file name of the uploaded run manifest, if applicable
Custom Add-On Protein Panel	If applied to a Teton run, lists the name of the custom protein panel
DISS Workflow	For a Teton Atlas run, lists the Teton Custom Atlas Workflow name
Description	A description of the run (optional)
Advanced	If applicable, advanced run settings for the run, such a custom recipe

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

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

   —A warning alerts you to expired consumables. Although not supported, AVITI OS allows the run to proceed.—
3. Select **Run** to start the run.
4. [Optional] If you imported run manifests from a USB drive, disconnect the USB drive.
5. Process the materials removed from the reagent bay:
   - » If you removed a used cartridge and buffer bottle, follow the instructions in *Discard the Cartridge and Bottle* on page 41.
   - » If you removed a wash tray, follow the guidelines for wash tray maintenance in the user guide for your instrument.

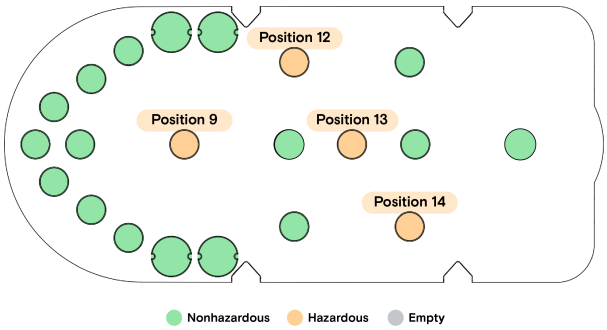
Monitor Run Metrics

1. Select **Overview** or **Details** to toggle between views of run details.
2. Monitor run metrics as they appear onscreen. AVITI OS indicates the expected batch during which metrics appear.
   —Expected cycles are approximate, and all metrics are estimates.—
3. Continue monitoring the run as AVITI OS refreshes the metrics.
4. When the run is complete, leave all materials on the instrument.
   - » To return to the Details view, select **Overview**.
   - » To access run data, go to your storage location.

Discard the Cartridge and Bottle

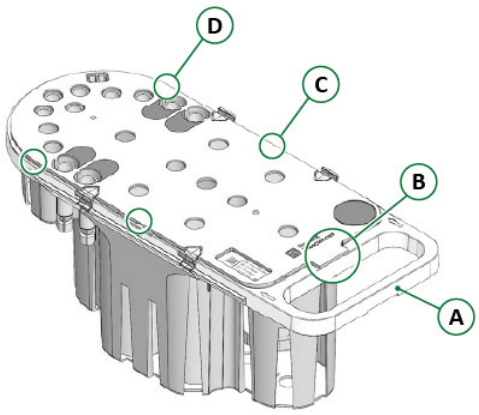
The cartridge and buffer bottle contain reagents with region-specific disposal requirements, which are described in the Safety Data Sheets (SDS) at elementbiosciences.com/resources.

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

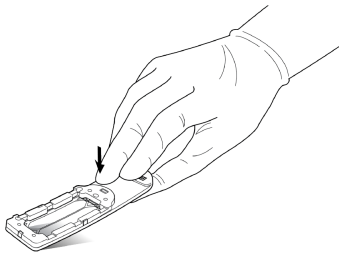
3. Remove the hazardous wells from the cartridge.
   —The volume remaining in each well depends on the number of cycles performed.—
4. Using a pipette tip or a similar tool, enlarge the hole in each foil seal to form a triangle.

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 nuclease-free water and dry upside down.

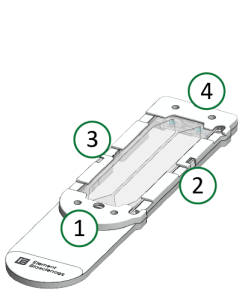
Flow Cell Recovery

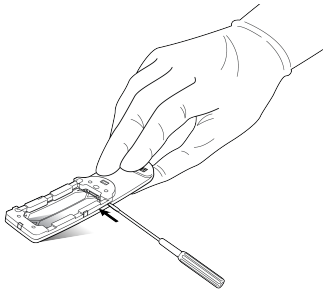
If the Flow Cell Integrity Test fails during run setup, perform the following steps to recover the flow cell.

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

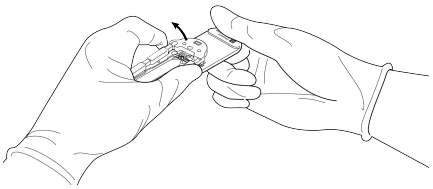


    b. Starting with position 1 below the slide area, insert a flat and rigid tool, such as a small screwdriver, into the gap along the side of the cartridge and toward the snap location. Maintain pressure on the cartridge and gently rotate the tool to release the snap.

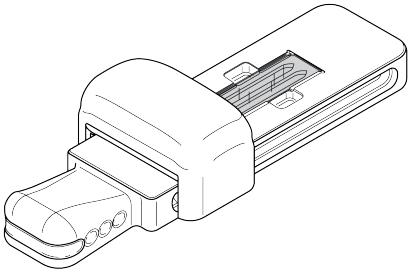




    c. With position 1 released, insert the tool at positions 2 and 3 along the side of the cartridge. Gently rotate the tool to release each snap.
    d. To release the snap at position 4, rotate the top half of the cartridge upward or use the tool from the side of the cartridge.



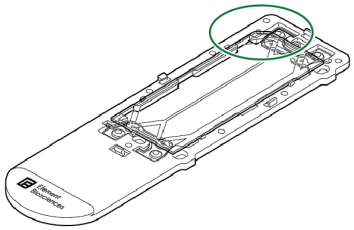
4. Lift to remove the slides from the bottom half of the flow cell cartridge.
5. Visually inspect the flow cell gaskets to make sure each gasket is well-seated.
6. Re-seal the slides using the flow cell sealer:
    a. Make sure the surface of the Teton flow cell sealer is clean. Thoroughly wipe the recessed 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.

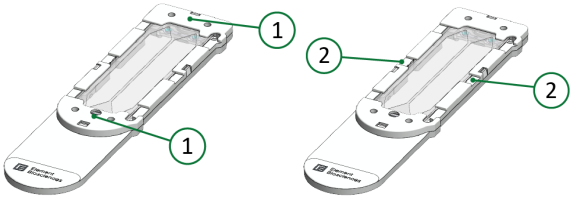
7. Place the sealed slides onto the bottom half of the flow cell cartridge with the beveled corner in the top-left position. Make sure the slides rest flat on the cartridge bottom.



8. Align the top half of the flow cell cartridge over the bottom half.
9. Press down on the cartridge 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 cartridge:

» Make sure there is no damage to the plastic and that the flow cell cartridge rests flat on a flat surface.

» Make sure there are no gaps along the sides of the cartridge top and bottom.

11. Wipe the assembled flow cell surface with an ethanol wipe and dry the surface with a lens wipe.
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.

CHAPTER 8

Consumables and Tools

This section lists available Teton kits and 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.

For Teton optimization and screening kits, see the *Teton Optimization & Screening User Guide (MA-00078)*.

Teton Cartridge and Reagent Kits

Teton—Part Number and Kit Name		Shipping	Storage
860-00039	Teton Cartridge and Reagent Kit [1]		
	– Teton Cartridge (820-00036)	-25°C to -15°C	-25°C to -15°C
	– Teton Reagent Kit (830-00055)	-25°C to -15°C	-25°C to -15°C
	– AVITI Buffer Bottle (Universal Wash Buffer) (820-00002)	Room temperature	Room temperature

[1] For use with *Teton Fixed Panels*, *Teton Full Custom Protein Panel*, and *Teton Focus Protein Panels*.

Teton Atlas—Part Number and Kit Name		Shipping	Storage
860-00040	Teton Atlas Cartridge and Reagent Kit - Low Output [2]		
	– Teton Atlas Low Output Cartridge (820-00039)	-25°C to -15°C	-25°C to -15°C
	– Teton Reagent Kit (830-00055)	-25°C to -15°C	-25°C to -15°C
	– AVITI Buffer Bottle (Universal Wash Buffer) (820-00002)	Room temperature	Room temperature
830-00056	Teton Atlas RNA Spike-In	-25°C to -15°C	-25°C to -15°C

[2] For use with the *Teton Atlas Low Output Protocol*, *Teton Full Custom Protein Panel*, and *Teton Focus Protein Panels*.

Teton Fixed Panel Kits

One fixed panel kit is required when using a Teton cartridge. Each fixed panel kit includes an RNA panel tube and protein panel tube.

Part Number and Kit Name		Shipping	Storage
830-00038	Teton Human Neuro Panel Kit	-25°C to -15°C	-25°C to -15°C
830-00039	Teton Human Immuno Panel Kit	-25°C to -15°C	-25°C to -15°C
830-00040	Teton Human MAPK-Cell Cycle Panel Kit	-25°C to -15°C	-25°C to -15°C
830-00041	Teton Human MAPK-Apoptosis Panel Kit	-25°C to -15°C	-25°C to -15°C

Teton Custom Add-On Protein Panel Kits

See *Teton Full Custom Protein Panel* on page 19.

Part Number and Kit Name		Shipping	Storage
860-00036	Teton Custom Add-On Protein Panel Assembly Kit		
	– Teton Custom Add-On Protein Panel Kit (830-00042)	-25°C to -15°C	-25°C to -15°C
	– Teton Custom Add-On Protein Buffer (830-00044)	-25°C to -15°C	-25°C to -15°C
830-00043	Teton Diversity Spike-In (8 reactions)	-25°C to -15°C	-25°C to -15°C

Teton Focus Protein Panels

See *Teton Focus Protein Panels* on page 22.

Part Number and Panel Name		Theme	Shipping	Storage
830-00046	Teton Focus Protein Panel, Set 1, Cytokine Signaling	Immunology	-25°C to -15°C	-25°C to -15°C
830-00047	Teton Focus Protein Panel, Set 2, T Cell Activation	Immunology	-25°C to -15°C	-25°C to -15°C
830-00048	Teton Focus Protein Panel, Set 3, Innate Immunity	Immunology	-25°C to -15°C	-25°C to -15°C
830-00049	Teton Focus Protein Panel, Set 1, Cell Metabolism	Cell biology	-25°C to -15°C	-25°C to -15°C
830-00050	Teton Focus Protein Panel, Set 2, Cell Stress & Apoptosis	Cell biology	-25°C to -15°C	-25°C to -15°C
830-00051	Teton Focus Protein Panel, Set 3, Gene Regulation	Cell biology	-25°C to -15°C	-25°C to -15°C
830-00052	Teton Focus Protein Panel, Set 1, Neurodegeneration	Neuroscience	-25°C to -15°C	-25°C to -15°C
830-00053	Teton Focus Protein Panel, Set 2, Neurodevelopment	Neuroscience	-25°C to -15°C	-25°C to -15°C
830-00054	Teton Focus Protein Panel, Set 3, Neuroinflammation	Neuroscience	-25°C to -15°C	-25°C to -15°C

Part Number and Kit Name		Shipping	Storage
830-00044	Teton Custom Add-On Protein Buffer	-25°C to -15°C	-25°C to -15°C

Teton Slide Kits

Part Number and Kit Name		Quantity	Shipping	Storage
860-00041	Teton Slide Kit, PLL – 48 Well (2-pack)	2	Room temperature	2°C to 8°C
860-00031	Teton Slide Kit, PLL – 12 Well (2-pack)	2	Room temperature	2°C to 8°C
860-00029	Teton Slide Kit, PLL – 1 Well (2-pack)	2	Room temperature	2°C to 8°C
860-00042	Teton Slide Kit, Uncoated – 48 Well (2-pack)	2	Room temperature	Room temperature
860-00032	Teton Slide Kit, Uncoated – 12 Well (2-pack)	2	Room temperature	Room temperature
860-00030	Teton Slide Kit, Uncoated – 1 Well (2-pack)	2	Room temperature	Room temperature

Teton Flow Cell Assembly Kits

Part Number and Kit Name		Quantity	Shipping	Storage
860-00028	Teton Flow Cell Assembly Kit, 12 Well (2-pack)	2	Room temperature	Room temperature
860-00027	Teton Flow Cell Assembly Kit, 1 Well or 48 Well (2-pack)	2	Room temperature	Room temperature

Teton Tools

Part Number and Kit Name		Quantity
860-00033	Teton Flow Cell Assembly Tool Set – Teton Flow Cell Aligner (810-00016) – Teton Flow Cell Sealer (810-00017) – Teton Slide Kit Tool (810-00021)	 1 1 1
860-00044	Teton Slide Kit Tray (2 pack)	2

User-Supplied Consumables

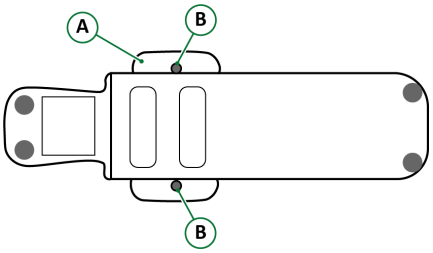
Consumable	Supplier
Biological-grade/RNase-free water	General lab supplier
C-Chip cell counting chamber slides	InCyto, catalog # DHC-N01
Cell culture medium appropriate for cell line	General lab supplier
Compressed air duster	General supplier
Dulbecco's Phosphate Buffered Saline (DPBS), with calcium, magnesium, 1X, pH 7–7.4	ThermoFisher Scientific, catalog # 14040117
Ethanol (EtOH), biological grade	General lab supplier
Ethanol wipes	General lab supplier
Formaldehyde	General lab supplier
Lens wipes	General lab supplier
Microseal 'B' adhesive seals, or equivalent	Bio-Rad, catalog # MSB1001
Phosphate-buffered saline (PBS), 1X, pH 7–7.4	General lab supplier
Pipette tips	General lab supplier
Pipettes, 16-channel, for 48-well slide kits Pipettes, 8-channel, for 12-well slide kits Pipette, single, for 1-well slide kits	ThermoFisher Scientific, catalog # TS 4662090 General lab supplier General lab supplier
Reagent trough	General lab supplier
RiboLock RNase Inhibitor	ThermoFisher Scientific, catalog # EO0381
Water bath float	General lab supplier
For use with custom add-on protein protocol: 96-well plates or 384-well plates 0.5 ml low-bind tubes 2 ml low-bind tubes	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_2O	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

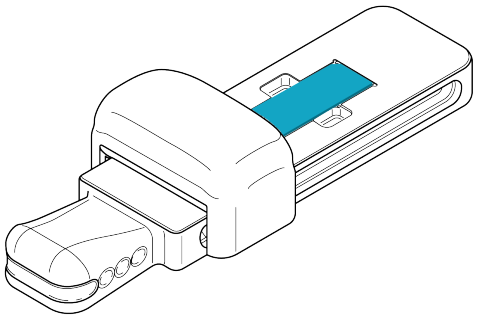
Caring for the Teton Flow Cell Sealer

- 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, disassemble and clean under the roller grip.
  - » 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.
  - » From the top of the sealer, use an ethanol 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. Add the appropriate volume of RiboLock RNase Inhibitor and 1X PBS to result in a 0.1 U/µl solution:

	48-Well or 12-Well Slide Kit	1-Well Slide Kit
RiboLock RNase Inhibitor	6 µl	7.5 µl
1X PBS	2.4 ml	3 ml

5. Add the appropriate volume of 1X PBS with 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	3 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.

Document History

Revision	Description of Change
December 2025 Document # MA-00053 Rev. F	• Updated Expanded Z and Small Cell Option language under Define Manual Run Parameters section. • Updated a step in fixation protocol for suspension cells to remove fixation reagent before the wash step. • Updated Teton Flow Cell Assembly cartridge to Teton Flow Cell Assembly Cassette to minimize confusion with Teton Cartridge. • Aligned RiboLock RNase inhibitor recommendations for adherent and suspension cells.
September 2025 Document # MA-00053 Rev. E	• Updated Teton Atlas cartridge, low output workflow and protocol to include protein panel as required instead of optional. • Reordered run preparation and setup steps to ensure flow cell is assembled after the priming step is complete. • Added additional guidance for matrigel surface coating. • Added updated drawing of slide tool kit for clarifying the disassembly step. • Updated User-Supplied Consumables to add an additional matrigel catalog number.
July 2025 Document # MA-00053 Rev. D	• Added descriptions of custom protein panel options. • Added Teton Focus Protein Panels and instructions for use, including an optional 24-plex custom protein panel protocol. • Added Teton Atlas cartridge, low output, and instructions for use. • Added 48-well slide kits and volumes for associated protocols. • Added the Teton slide kit tool and instructions for use. • Updated kit descriptions in Overview chapter. • Updated instructions for preparing suspension cells on a 1-well slide kit. • Updated run setup steps when using AVITI OS v3.4. • Updated 860-00027 Teton Flow Cell Assembly kit name to 1 well or 48 well. • Updated steps to dispose reagents to show three main points of resistance when removing the cartridge lid. • Removed the small cell option from run setup steps. • Removed optional permeabilization step from run preparation. • Removed the optimization kit. See the *Teton Optimization & Screening User Guide (MA-00078)*.

Revision	Description of Change
April 2025 Document # MA-00053 Rev. C	• Restructured guide with chapter headings to better organize workflow and add-on protocols. • Added fixed panel RNA and protein tubes to Prepare Reagents section. • Added instructions for attaching and fixing suspension cells. • Added Teton Cartridge and Reagent Kits and Teton fixed panel kits. • Added instructions for adding fixed panel tubes to the cartridge during run setup. • Added Teton Custom Add-On Protein Panel Assembly Kit and Teton Diversity Spike-In and instructions for use. • Added Universal Wash Buffer to AVITI Buffer Bottle in kit component list. • Updated kit descriptions in Overview chapter. • Updated run setup steps when using AVITI OS v3.3. • Updated expected priming time to 24 minutes. • Updated concentration of matrigel solution to 0.1–0.25 mg/ml. • Updated fixation reagent to 8% formaldehyde.
February 2025 Document # MA-00053 Rev. B	• Added well thumbnail images to show successful and unsuccessful results. • Added diagrams to sample preparation to emphasize proper pipette placement when adding or removing liquid from wells. • Reordered run preparation and setup steps to ensure flow cell is assembled before confirming flow cell assembly on the AVITI OS interface. • Updated step in fixation protocol to remove liquid from final wash before adding RNase inhibitor and storing fixed cells. • Updated microscope filter specifications to include nucleus (cy5) and membrane (cy3). • Updated volume of Teton optimization reagent from 80 µl to 70 µl. • Updated 1X PBS wash volume to 200 µl and 2 ml regardless of whether samples were stored before adding Teton reagents. • Updated note for optional permeabilization step to emphasize that the Element protocol does not require permeabilization.
December 2024 Document # MA-00053 Rev. A	• Initial release.

Technical Support

Visit the 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.

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