Element Biosciences

Teton™ Optimization & Screening

User Guide

FOR USE WITH

AVITI24™ System
AVITI Operating Software v3.4 or later
Teton Custom Antibody Screening Kit
Teton Onboard Cell Paint Imaging Kit
Teton Cell Paint Probe Kit

ELEMENT BIOSCIENCES
For Research Use Only. Not for Use in diagnostic procedures.

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CHAPTER 1

Overview

This guide includes workflows for antibody screening using the Teton Custom Antibody Screening Kit and cell culture optimization using the Teton Onboard Cell Paint Imaging Kit or Teton Cell Paint Probe Kit.

The Teton Custom Antibody Screening Kit screens compatible primary antibodies for optimal results before using the Teton Custom Add-On Protein Panel Assembly Kit to prepare a full custom protein panel. The workflow requires prepared cell samples on a Teton Slide Kit and primary antibodies of interest. The Teton Custom Antibody Screening Kit prepares samples for an antibody screening run on the AVITI24 System.

After culturing cells on a Teton Slide Kit, perform optimization of culture conditions such as cell growth and morphology using the Teton Cell Paint Probe Kit with a fluorescent microscope or Teton Onboard Cell Paint Imaging Kit with an AVITI24 System.

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

The following table lists the features and applications for these three optimization and screening kits:

Kits	Teton Custom Antibody Screening Kit	Teton Onboard Cell Paint Imaging Kit	Teton Cell Paint Probe Kit
Teton Cartridge	Teton Custom Screen Cartridge	Teton Custom Screen Cartridge	Not included
Applications	• Cell morphology • Cell paint images • Cell counts • Confluency • Segmentation • Custom primary antibody screening • Custom primary antibody localization • Expression relative to control	• Cell morphology • Cell paint images • Cell counts • Confluency • Segmentation	• Cell morphology • Cell paint images
Teton Slide Kit	48-well or 12-well compatible	48-well or 12-well compatible	48-well or 12-well compatible
Imaging	AVITI24 System	AVITI24 System	Fluorescent microscope
AVITI24 Run time	4 hours	3 hours	Not applicable
AVITI OS Assay Options	• Cell Paint + Control Antibodies + Custom Antibodies • Cell Paint + Control Antibodies • Cell Paint Only	• Cell Paint Only	Not applicable

Teton Custom Antibody Screening Kit

The Teton Custom Antibody Screening Kit is designed for screening custom primary antibodies on an AVITI24 System to confirm compatibility with the Teton Custom Add-On Protein Panel Assembly Kit. The kit enables screening of two custom primary antibodies per well. Screen up to 24 custom antibodies on a 12-well Teton Slide Kit or up to 96 custom antibodies on a 48-well Teton Slide Kit without requiring a full 24-hour cytoprofiling run.

The Teton Custom Antibody Screening Kit also enables testing regulation of custom primary antibodies and comparisons across specific cell lines. See the *Teton Custom Antibody Screening Technical Note (LT-00056)*.

The antibody screening run provides a readout of target counts per cell for controls and custom primary antibodies along with the cell morphology output including confluency, cell area, cell diameter and CellProfiler values. Comparison of the custom primary antibody counts relative to the negative control counts assists in determining if the custom primary antibodies are compatible with the Teton Custom Add-On Protein Panel Assembly Kit.

The Teton Custom Antibody Screening Kit includes the following reagents that allow for one custom antibody screening run:

- Teton Custom Screen Cartridge and AVITI Buffer
- Teton Custom Antibody Screen Probe Kit with coordinating molecules for custom primary antibodies, positive and negative controls, and a dilution buffer
- Teton Cell Paint Probe Kit with cell paint probes for visualization and a wash buffer

Depending on the type of screening that is planned, use the following options to design the antibody screening assay and select the appropriate assay on AVITI OS:

1. To screen for custom antibody compatibility and sub-cellular localization in specific cell lines using the antibody screening protocol, see *Teton Custom Antibody Screening* on page 15. Select the Cell Paint + Control Antibodies + Custom Antibodies assay option during the AVITI OS run setup.
2. To confirm compatibility of specific cell lines with Teton cell paint and protein probes using the Teton Custom Antibody Screening Kit without any custom primary antibodies, see *Prepare Teton Antibody Pool* on page 19. Select the Cell Paint + Control Antibodies assay option during the AVITI OS run setup.
3. To test surface compatibility, and check for cell morphology, confluency and proper sample preparation technique, use only the Teton Cell Paint Probe Kit. Select the Cell Paint only assay option during the AVITI OS run setup.

Teton Onboard Cell Paint Imaging Kit

The Teton Onboard Cell Paint Imaging Kit allows cell culture optimization after preparing cell samples on a 48-well or 12-well Teton Slide Kit. The sample assessment is performed on an AVITI24 System.

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

With the Teton Onboard Cell Paint Imaging Kit, you can test up to 48 different cell lines or cell culture conditions in a single cell culture optimization run to test compatibility of these experimental conditions with the Teton run.

The Teton Onboard Cell Paint Imaging Kit includes the following reagents that allow for one cell culture optimization run:

- Teton Custom Screen Cartridge and AVITI Buffer
- Teton Cell Paint Probe Kit with cell paint probes for visualization and a wash buffer

Teton Cell Paint Probe Kit

The Teton Cell Paint Probe Kit allows optimization of sample preparation quality, cell growth, and morphology after preparing cell samples on a 48-well or 12-well Teton Slide Kit. The sample assessment is performed using a fluorescent microscope.

NOTE
If the Teton Cell Paint Probe Kit is used as part of the Teton Custom Antibody Screening Kit or Teton Onboard Cell Paint Imaging Kit, the assessment is performed on an AVITI24 System.

The Teton Cell Paint Probe Kit includes the following reagents:

- Teton Optimization Cell Paint Probes for visualization
- Teton Optimization Wash

Optimization and Screening Run Consumables

An antibody screening run or cell culture optimization run on the AVITI24 System requires one each of the following Teton kits:

- Teton Custom Antibody Screening Kit for antibody screening or Teton Onboard Cell Paint Imaging Kit for cell culture optimization
- Teton Slide Kit (48-well or 12-well)
- Teton Flow Cell Assembly Kit

The following Teton tools are required for assembling the Teton flow cell:

- Teton Flow Cell Assembly Tool Set
- Teton Slide Kit Tool

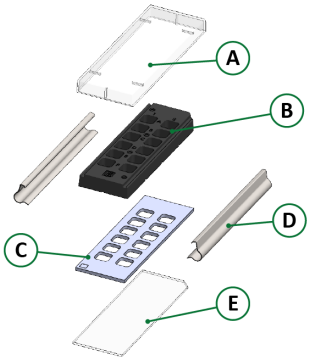
For a list of kits with catalog numbers, see *Consumables and Tools* on page 43.

Teton Custom Screen Cartridge

A Teton Custom Screen Cartridge is used for an antibody screening run or cell culture optimization run on an AVITI24 System. The cartridge includes required reagents for one run.

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 cytoprofiling run, the slide is reassembled as a flow cell with parts from the Teton Flow Cell Assembly Kit.

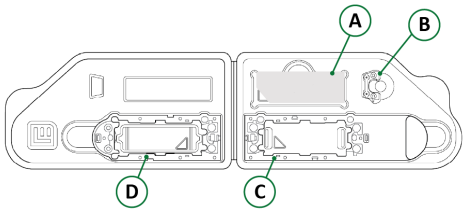


A Lid
B Frame
C Gasket
D Side clip
E Glass slide

Slide kits are available in 48-well or 12-well configurations, and for each configuration as PLL-coated or uncoated surfaces.

Teton Flow Cell Assembly Kit

The Teton Flow Cell Assembly Kit contains an adhesive slide, two flow cell gaskets, and the top and bottom cartridge parts. Before the run, the sample slide is assembled and packaged into a Teton flow cell using the provided components. See *Assemble the Teton Flow Cell* on page 28.



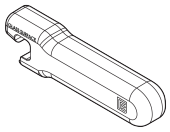
A Adhesive slide
B Flow cell gaskets (2)
C Cartridge bottom
D Cartridge top

One flow cell assembly kit is required to load one sample slide for an antibody screening run.

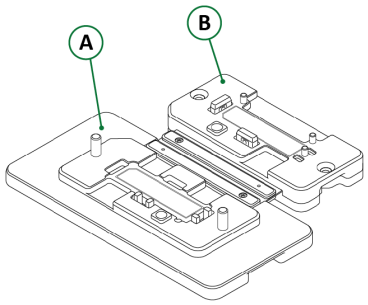
Teton Flow Cell Assembly Tools

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 28.

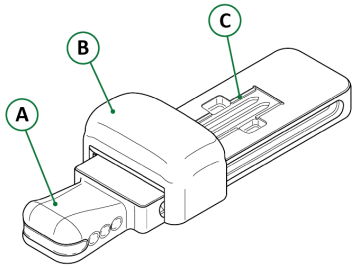


- **Teton Flow Cell Aligner**—The Teton flow cell aligner aligns and adheres the sample slide from the slide kit to the adhesive slide provided in the Teton Flow Cell Assembly Kit. See *Align and Seal the Slides* on page 29.



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 the *Teton CytoProfiling User Guide (MA-00053)*.



A Base grip
B Roller grip
C Indentation for slides

CHAPTER 2

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.

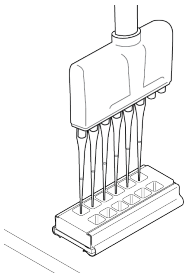
- *Culture and Fix Adherent Cells* on page 11
- *Attach and Fix Suspension Cells* on page 13

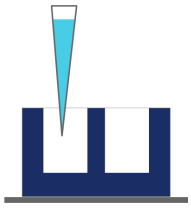
NOTE
Cells expressing fluorescent proteins can interfere with Teton readouts and are not recommended.

To determine the success of sample preparation, use the Teton Cell Paint Probe Kit (part # 830-00035) to view samples on a microscope, or use the Teton Onboard Cell Paint Imaging Kit (part # 860-00047) to view samples after a short run on the AVITI24 System.

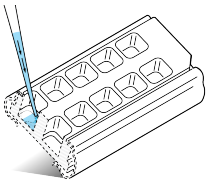
Protocol Guidelines

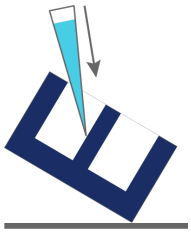
- **Perform sample preparation steps in a biosafety cabinet.** Steps involving live cells ***must*** be performed in a biosafety cabinet.
- Avoid disturbing the slide surface throughout the protocol.
- Do not allow cells to dry out. Allowing cells to dry out can result in cell detachment.
- Do not fix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



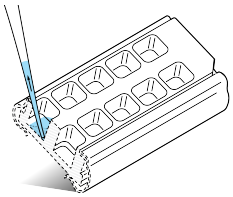


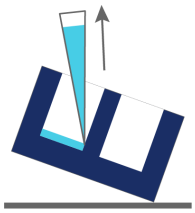
- When dispensing cells, hold the pipette perpendicular to the glass slide surface and dispense into the center of each well.
- Do not contact the slide surface.
- Do not swirl the pipette when loading.
- To reduce performance variability, ensure the slide kit is on a flat and level surface during cell 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 slide kits at the same time to avoid cells from drying out during the 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
50 µl	200 µl

NOTE
For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpipette. See *User-Supplied Consumables* on page 44.

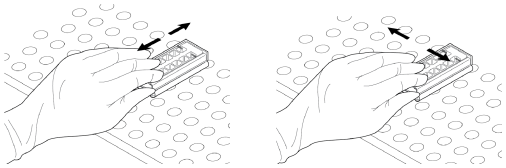
5. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
6. Repeat the wash one time.
7. Ensure the cells are fully dissociated to single cells and counted.
8. With the slide kit on a flat and level surface, hold the pipette 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
40 µl	150 µl

Use the following information to estimate initial cell seeding density.

	48-Well Slide Kit	12-Well Slide Kit
Well size:	3.5 mm x 3.5 mm	7 mm x 7 mm
Element uses:	2.4–2.8 K HeLa cells per well	9–10 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 50–70% in each well. Do not allow cell overgrowth.

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

Fix Cultured Adherent Cells

1. Gather the following consumables:
   - 1X Dulbecco's Phosphate Buffered Saline (DPBS), pH 7–7.4, sterilized
   - 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
50 µl	200 µl

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
40 µl	150 µl

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
50 µl	200 µl

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:
    - Perform a Custom Antibody Screening run on the AVITI24 System. See *Teton Custom Antibody Screening* on page 15.
    - Assess success of sample preparation. See *Teton Cell Culture Optimization* on page 22.

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 slide kits at the same time to avoid cells from drying out during the process.

Attach Suspension Cells

1. Gather the following consumables:
   - » 1X Phosphate Buffered Saline (PBS), pH 7–7.4
   - » Teton Slide Kit
   - » 15 ml or 50 ml Falcon tube
2. To ensure PBS remains sterile, always open the PBS 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 PBS 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.

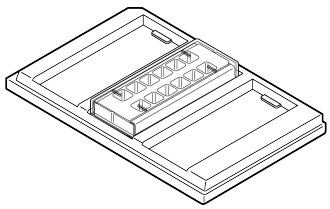
48-Well Slide Kit	12-Well Slide Kit
40 µl	150 µl

NOTE
For the 48-well slide kit, use a P-200 multichannel pipette or 16-channel Finnpipette. See *User-Supplied Consumables* on page 44.

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 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

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
   - » (Optional) 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
40 µl	150 µl

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. 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
50 µl	200 µl

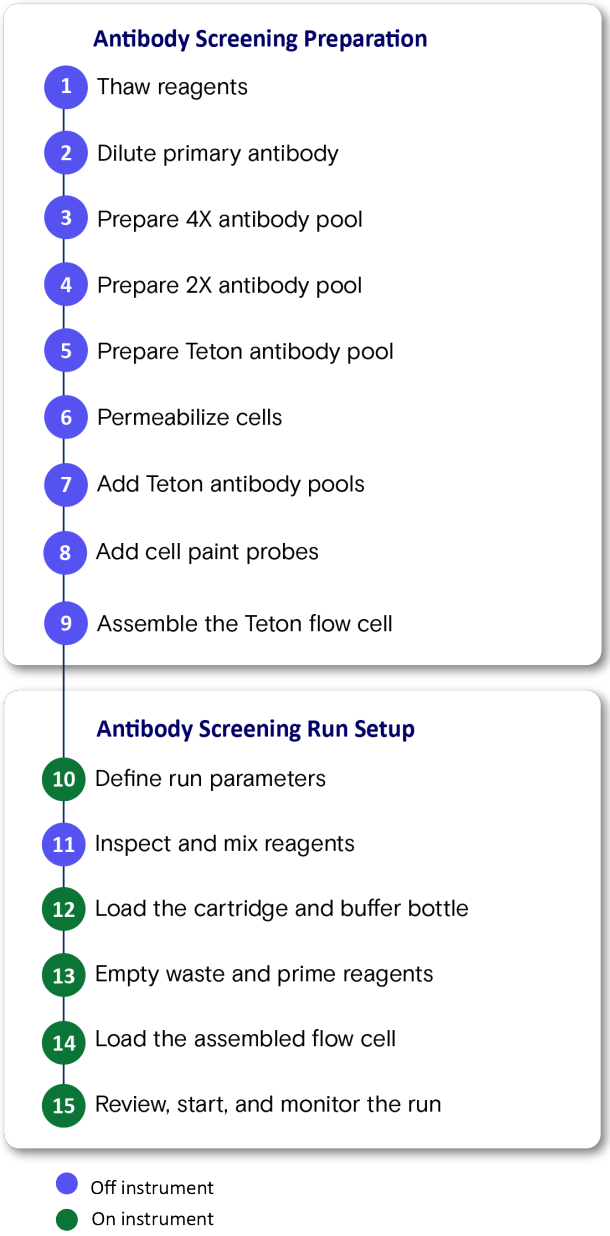
7. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
8. Repeat the wash two more times. Do not remove the liquid after the final wash.
9. After fixing cells, proceed to one of the following options:
   - » Perform a Custom Antibody Screening run on the AVITI24 System. See *Teton Custom Antibody Screening* on page 15.
   - » Assess success of sample preparation. See *Teton Cell Culture Optimization* on page 22.

CHAPTER 3

Teton Custom Antibody Screening

Use the Teton Custom Antibody Screening Kit to assess antibody compatibility, sample quality, cell growth, and morphology. After sample preparation on a 48-well or 12-well slide kit, perform an antibody screening run on the AVITI24 System.

Protocol Summary



Prerequisites

1. Confirm that primary antibodies are compatible.

   NOTE
   Only rabbit antibodies are compatible with this protocol. Non-rabbit host custom primary antibody can cause false positive results.

   - For optimal performance, use monoclonal antibodies
   - Select custom primary antibodies that are validated by Western blot and immunocytochemistry/immunofluorescence or flow cytometry for reliable and consistent results with the Teton assay

2. Prepare cell samples on a 48-well or 12-well slide kit. See *Sample Preparation* on page 10.
3. Determine plexity based on the total number of custom primary antibodies that you plan to screen with this protocol.

Prepare Reagents

Preparing reagents for a custom antibody screening run requires thawing the Teton Custom Screen Cartridge, Teton Cell Paint Probe Kit, and Teton Custom Antibody Screen Probe Kit followed by preparing Teton antibody pools.

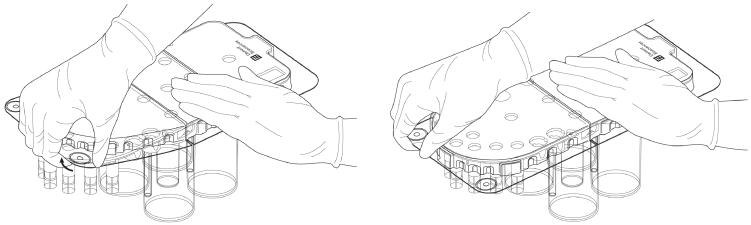
Thaw Reagent Cartridge

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 Antibody Screening Reagents

1. When the reagent cartridge has been in the water bath for 2 hours, gather the following reagents from -25°C to -15°C storage:
   - Teton Custom Antibody Screen Control
   - Teton Custom Antibody Screen Protein 1

» Teton Custom Antibody Screen Protein 2
» Teton Custom Antibody Screen Dilution Buffer
» Teton Optimization Cell Paint Probes
» Teton Optimization Wash

2. Thaw the above consumables in a room temperature water bath for 15 minutes.
3. Invert each tube 10 times to mix and then place on ice until use.

Prepare Antibody Pool and Cell Samples

Use up to two custom primary antibodies per well with the screening kit. Prepare all antibody pool mixes on the day of use.

Preparing the antibody pool involves diluting the custom primary antibodies at a required concentration. Combine diluted custom primary antibodies with Teton Custom Antibody Screen Protein to form custom primary antibody probes. Mix the custom primary antibody probes with Teton Custom Antibody Screen Control, which contains a positive and a negative control to form Teton antibody pools.

Depending on the type of screening that is planned, prepare the antibody pool and select the appropriate assay on AVITI OS as follows:

- To screen for custom antibody compatibility and cellular localization in specific cell lines using the antibody screening protocol, prepare the antibody pool with Teton Custom Antibody Screen Controls, Teton Custom Antibody Screen Proteins and custom primary antibodies. See *Teton Custom Antibody Screening* on page 15). Select the **Cell Paint + Control Antibodies + Custom Antibodies** assay option during the AVITI OS run setup.
- To confirm compatibility of specific cell lines with Teton cell paint and protein probe, prepare the antibody pool with Teton Custom Antibody Screen Controls and Teton Custom Antibody Screen proteins. Do not add custom primary antibodies to the antibody pool. See *Prepare Teton Antibody Pool* on page 19. Select the **Cell Paint + Control Antibodies** assay option during the AVITI OS run setup.
- To test surface compatibility of the slide kit with specific cell lines, and check for cell morphology, confluency and proper sample preparation technique, use only the Teton Cell Paint Probe Kit without any custom primary antibodies or Teton Custom Antibody Screen Controls. See *Add Teton Cell Paint Probe Reagents* on page 23. Select the **Cell Paint only** assay option during the AVITI OS run setup.

Preparing for the antibody screening run includes incubating cell samples with Teton antibody pools and Teton Optimization Cell Paint Probes followed by assembling the Teton Flow Cell.

Example Experimental Setup

To ensure successful identification of usable custom primary antibodies for the Teton run, test multiple custom primary antibodies to the same target. Randomizing the sample setup helps in minimizing any performance bias due to well position on the flow cell.

This table shows an example of an experimental setup for a 12-well slide kit with varying combinations of custom primary antibody vendors and lot numbers using 2 different cell lines. Column 2 is a duplicate of Column 1 to allow for testing in replicates.

Well	1	2
A	**Cell Line A** Custom Antibody 1: Vendor #1; Lot #1 Custom Antibody 2: Vendor #1; Lot #1	**Cell Line A** Custom Antibody 1: Vendor #1; Lot #1 Custom Antibody 2: Vendor #1; Lot #1
B	Custom Antibody 1: Vendor #1; Lot #2 Custom Antibody 2: Vendor #1; Lot #2	Custom Antibody 1: Vendor #1; Lot #2 Custom Antibody 2: Vendor #1; Lot #2
C	Custom Antibody 1: Vendor #2; Lot #1 Custom Antibody 2: Vendor #2; Lot #1	Custom Antibody 1: Vendor #2; Lot #1 Custom Antibody 2: Vendor #2; Lot #1
D	**Cell Line B** Custom Antibody 1: Vendor #1; Lot #1 Custom Antibody 2: Vendor #1; Lot #1	**Cell Line B** Custom Antibody 1: Vendor #1; Lot #1 Custom Antibody 2: Vendor #1; Lot #1
E	Custom Antibody 1: Vendor #1; Lot #2 Custom Antibody 2: Vendor #1; Lot #2	Custom Antibody 1: Vendor #1; Lot #2 Custom Antibody 2: Vendor #1; Lot #2
F	Custom Antibody 1: Vendor #2; Lot #1 Custom Antibody 2: Vendor #2; Lot #1	Custom Antibody 1: Vendor #2; Lot #1 Custom Antibody 2: Vendor #2; Lot #1

Prepare 4X Custom Antibody Pool

Use the volumes listed below as a guide while preparing the antibody mix.

You can use a 96-well plate or 384-well plate and a multichannel pipette with the 12-well or 48-well Teton slide kits, respectively to perform antibody dilutions and pooling. If you are preparing larger volumes for antibody dilutions and pooling, you can use low-bind tubes.

1. Thaw each custom primary antibody, if stored frozen.
2. Invert the antibody tube 10 times to mix. Briefly centrifuge the tube and pipette to mix.
3. Perform serial dilutions of your custom primary antibody in PBS as needed to achieve a stock concentration of 16 nM.

   If you used plates for primary antibody dilutions, briefly centrifuge the plate for 1–2 minutes to remove any bubbles.
4. Use the Teton Custom Antibody Screen Dilution Buffer to dilute each of the custom primary antibody to 24 ng/ml (0.16 nM).
5. Briefly centrifuge Teton Custom Antibody Screen tubes and pipette to mix contents in each tube.
6. Mix the diluted custom primary antibody 1 (24 ng/ml) with Teton Custom Antibody Screen Protein 1 (8X concentration) at a 1:1 ratio to prepare 4X Custom Antibody Mix 1.

   If you are screening two custom primary antibodies, mix the diluted custom primary antibody 2 (24 ng/ml) with Teton Custom Antibody Screen Protein 2 (8X concentration) at a 1:1 ratio to prepare 4X Custom Antibody Mix 2.

Reagent	48-Well Slide Kit	12-Well Slide Kit
Teton Custom Antibody Screen Protein	3.125 µl/well	10 µl/well
Diluted custom primary antibody	3.125 µl/well	10 µl/well
7. Pipette to mix. Do not vortex.
8. Incubate each antibody mix at room temperature for 1 hour. Store on ice until use.

Prepare 2X Custom Antibody Pool

Use the volumes listed below as a guide while preparing the antibody mix.

1. Combine 4X Custom Antibody Mix 1 and 4X Custom Antibody Mix 2 in a 1:1 ratio.

   If you are screening only a single custom primary antibody, prepare 2X Custom Antibody Pool by combining 4X Custom Antibody Mix 1 and Teton Custom Antibody Screen Dilution Buffer in a 1:1 ratio.

Reagent	48-Well Slide Kit	12-Well Slide Kit
4X Custom Antibody Mix 1	6.25 µl/well	20 µl/well
4X Custom Antibody Mix 2 **or** Teton Custom Antibody Screen Dilution Buffer	6.25 µl/well	20 µl/well

2. Pipette to mix. Do not vortex. Store on ice until use.

Prepare Teton Antibody Pool

Use the volumes listed below as a guide while preparing the antibody mix.

1. Combine 2X Custom Antibody Pool and 2X Teton Custom Antibody Screen Control in a 1:1 ratio.

   If you are not screening any custom primary antibodies and testing the compatibility of your specific cell lines with Teton cell paint and protein probes, prepare Teton antibody pool by combining Teton Custom Antibody Screen Control and Teton Custom Antibody Screen Dilution Buffer in a 1:1 ratio.

Reagent	48-Well Slide Kit	12-Well Slide Kit
Teton Custom Antibody Screen Control	12.5 µl/well	40 µl/well
2X Custom Antibody Pool **or** Teton Custom Antibody Screen Dilution Buffer	12.5 µl/well	40 µl/well

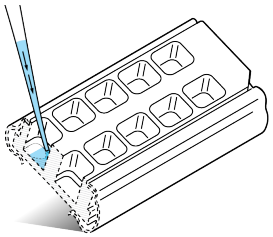
2. Pipette to mix. Do not vortex.
3. Store the Teton antibody pool on ice until use.
4. Proceed immediately to *Permeabilize Cells* on page 19 or store the Teton antibody pool at -25°C to -15°C for up to 24 hours if you plan to ship samples.

Permeabilize Cells

Perform all steps in a biosafety cabinet.

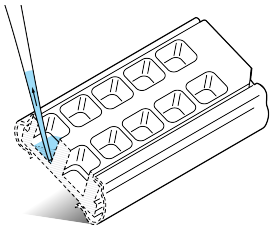
1. Prepare permeabilization reagent (70% ethanol)—Prepare fresh daily.
   - » 7 ml ethanol (EtOH), biological grade
   - » 3 ml biological-grade/RNase-free water
2. To remove liquid, slightly tip the slide kit and position the pipette tip in the corner of the well. Do not contact the slide surface.
3. Slightly tip the slide kit and slowly add the appropriate volume of permeabilization reagent along the middle of each well wall.

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



4. Cover the slide kit and incubate for 10 minutes at room temperature. Do not exceed a 10-minute incubation.
5. To wash the wells, slightly tip the slide kit and remove the appropriate volume of permeabilization reagent from each well. Then, slowly add the appropriate volume of 1X PBS along the middle of each well wall.

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



6. Repeat the wash three more times. Do not remove the liquid after the final wash.

Add Teton Antibody Pools

Perform all steps in a biosafety cabinet.

1. Pipette mix the Teton antibody pool stored on ice.

   If the Teton antibody pool was frozen, thaw the antibody pool in a room temperature water bath for 15 minutes and pipette mix.
2. 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.
3. Add appropriate volume of Teton antibody pool to each well. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.

48-Well Slide Kit	12-Well Slide Kit
25 µl	80 µl

   NOTE
   Use a reagent trough for convenient dispensing using a multichannel pipette with 48-well and 12-well slide kits.
4. Incubate at room temperature for 30 minutes.
5. Remove the Teton antibody pool from each well. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface. Make sure all liquid is removed.
6. Add appropriate volume of 1X PBS to each well. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.

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

7. Remove the 1X PBS from each well. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
8. Repeat the wash three more times.

Add Teton Cell Paint Reagents

1. Ensure Teton Cell Paint Probe Kit contents are thawed.
2. Invert each tube 10 times to mix and then briefly centrifuge. *Do not vortex*.
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. Slightly tip the slide kit and slowly add the appropriate volume of Teton Optimization Cell Paint Probes along the middle of each well wall.

48-Well Slide Kit	12-Well Slide Kit
25 µl	80 µl

NOTE
Fill volumes for Teton Optimization Cell Paint Probes supplied in the kit enable the use of a reagent trough and multichannel pipette with 48-well and 12-well slide kits.

5. Incubate at room temperature for 5 minutes.
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 Optimization Wash along the middle of the wall of each well.

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

8. Repeat the wash two more times. Do not remove the liquid after the final wash.
9. Proceed to *Run Preparation and Setup* on page 28.

CHAPTER 4

Teton Cell Culture Optimization

Use the Teton Cell Paint Probe Kit or Teton Onboard Cell Paint Imaging Kit to assess sample quality, cell growth, and morphology after sample preparation on a 48-well or 12-well slide kit. The Teton Cell Paint Probe Kit protocol prepares the sample slide for viewing under a fluorescent microscope while the Teton Onboard Cell Paint Imaging Kit protocol prepares the sample slide for imaging on an AVITI24 System.

Requirements

- Prepared cell samples on a 48-well or 12-well slide kit (see *Sample Preparation* on page 10)
- Slide holder adapter, Agilent BioTek, catalog # 1220548 (for use with Teton Cell Paint Probe Kit)

Microscope Specifications (applicable for Teton Cell Paint Probe Kit only)

Item	Specification
Microscope	Fluorescent
Filters	Green: 515–560 nm excitation, 580–650 nm emission (similar to Cy3 filter for cell membrane) Red: 620–650 nm excitation, 660–750 nm emission (similar to Cy5 filter for cell nucleus)
Objective	Any fluorescence objective that can image through 1 mm thick glass slide, such as Olympus UCPLFLN20X Higher NA objectives achieve better resolution and fluorescent signal
Laser Power & Settings	Targeted 30 mW power output, may vary depending on the instrumentation

Protocol Summary

Thaw Teton Cell Paint Reagents

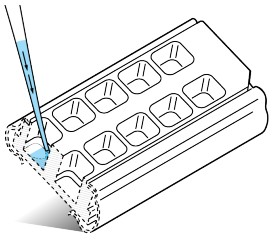
1. When the reagent cartridge is almost thawed, gather the following reagents from -25°C to -15°C storage:
   - » Teton Optimization Cell Paint Probes
   - » Teton Optimization Wash
2. Thaw the above consumables in a room temperature water bath for 15 minutes.
3. Invert each tube 10 times to mix and then place on ice until use.
4. If samples were stored after the fixation step, remove samples from 2°C to 8°C storage.

Add Teton Cell Paint Probe Reagents

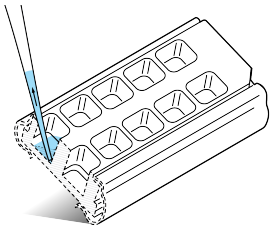
Perform all steps in a biosafety cabinet.

1. 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.
2. Wash each well with appropriate volume of 1X PBS. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.

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



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. Repeat the wash one more time. Pipette to dry each well before proceeding. Make sure no flowing liquid is observed.
5. Add appropriate volume of Teton Optimization Cell Paint Probes to each well. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.

48-Well Slide Kit	12-Well Slide Kit
25 µl	70 µl

NOTE
Fill volumes for Teton Optimization Cell Paint Probes provided in the Teton Cell Paint Probe Kit (part # 830-00045) enable the use of a reagent trough and multichannel pipette with 48-well and 12-well slide kits.

6. If using the Teton Cell Paint Probe Kit (830-00045), incubate at room temperature for 5 minutes. Do not exceed 5 minutes.

   If using the original optimization kit (860-00022), incubate at room temperature for 1 minute. Do not exceed 2 minutes.

   CAUTION
   Over-incubation can lead to saturation of signal in certain cell lines.
7. Remove the Teton Optimization Cell Paint Probes from each well. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface. Make sure all liquid is removed.
8. Add appropriate volume of Teton Optimization Wash Buffer to each well. Slightly tip the slide kit and slowly add liquid along the middle of each well wall.

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

9. Remove the Teton Optimization Wash from each well. Slightly tip the slide kit and position the pipette tip in the corner of each well. Do not contact the slide surface.
10. Repeat the wash two more times.
11. If using the Teton Onboard Cell Paint Imaging Kit, proceed to *Run Preparation and Setup* on page 28.

If using the Teton Cell Paint Probe Kit, proceed to *Prepare Slides for Microscopy* on page 25.

Prepare Slides for Microscopy

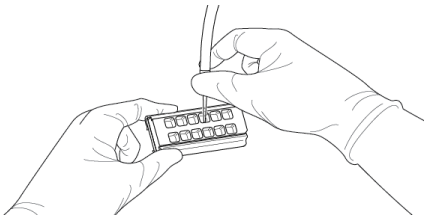
The slide kit is disassembled to remove the sample slide. The sample slide is sealed with an adhesive slide or cover slip and then loaded onto a fluorescent microscope for viewing and imaging the samples.

Gather the following tools, kits, and recommended equipment:

- Vacuum aspiration system with 200 µl tip (recommended)
- Teton slide kit tool
- Teton flow cell assembly kit or cover slip
- Optional Teton flow cell aligner and flow cell sealer

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.

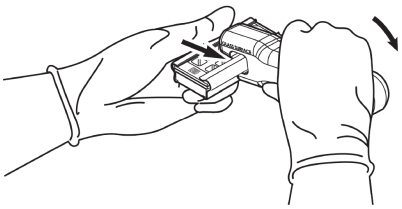


> Perform step **2** to remove the metal side clips using the Teton slide kit tool (part # 810-00021).
> Otherwise, perform step **3**.

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.

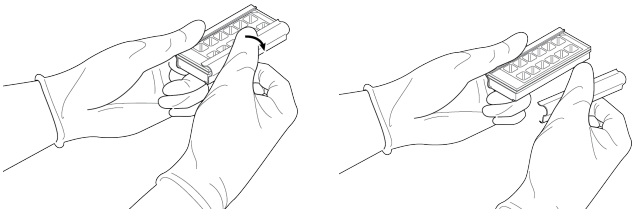


   d. Repeat step **2** to remove the metal side clip from the opposite side of the slide kit.

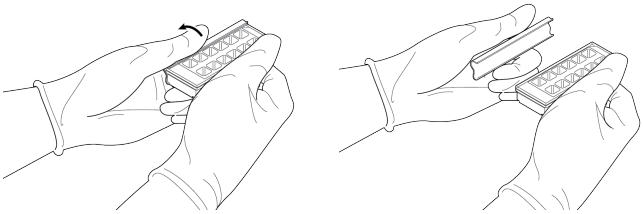
3. Remove metal side clips *using your fingers:*
   a. Turn the slide kit upside down so the open wells are facing downward and the glass slide is facing upward.

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.

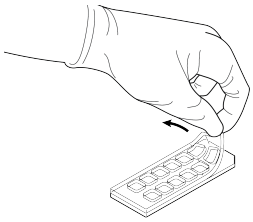
CAUTION
Avoid damage to the slide. Do not pull the side clip from the end regions of the clip. Always pull the clips from the center of each clip.



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.



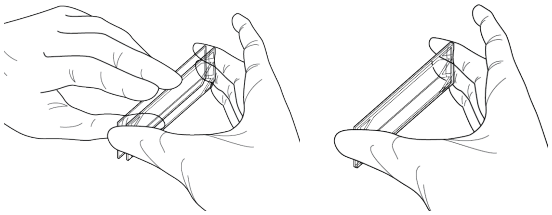
Assemble Sample Slide

The sample slide can be prepared for viewing on a microscope by applying a cover slip to the sample slide or manually assembling the sample slide with the adhesive slide provided in the Teton Flow Cell Assembly Kit.

To manually assemble slides by hand, you must carefully align the slides before allowing the adhesive slide to touch the sample slide. Misalignment can prevent the slides from fitting into the slide holder adapter.

> If you have a flow cell aligner, use the flow cell aligner to assemble the slides. See *Align and Seal the Slides* on page 29.

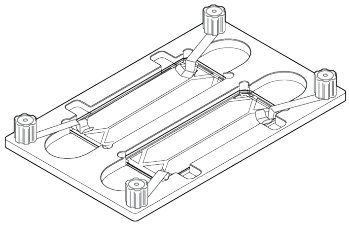
1. Remove the adhesive slide from the Teton Flow Cell Assembly Kit.
2. Starting from the beveled corner, peel off the protective film from the adhesive slide.
3. Hold the sample slide with one hand and the adhesive slide with the other hand. Make sure the beveled corners are aligned.
4. Using your fingers on the edges of each slide, align the two slides but do not allow them to touch. Work slowly to maintain alignment and allow the slides to come together.



5. Press to securely adhere the slides and remove any air.

Load Slides onto Microscope

1. Load slides onto a slide holder adapter.



For an inverted microscope:
- Place the beveled corner of the slides at the top-right side of the holder.
- In this position, cells are on the bottom surface for imaging.

For an upright microscope:
- Place the beveled corner of the slides at the top-left side of the holder.
- In this position, cells are on the top surface for imaging.

2. To adjust autofocus, consider a 1 mm thickness of the sample slide.
3. Collect 3–4 Z stack images with 2 µm spacing for optimal spatial resolution.

   For cells with a greater Z variance, increase stacks as necessary.

See *Run Results* on page 37 for example of expected results with some commonly used cell lines. See *Troubleshoot Sample Preparation* on page 40 for sample preparation issues.

CHAPTER 5

Run Preparation and Setup

Performing an antibody screening or cell culture optimization run on an AVITI24 System includes steps to assemble the flow cell and then follow prompts on the AVITI OS interface to setup the run.

Before run preparation and setup, ensure cells are prepared on a Teton slide kit and treated with Teton antibody pools and cell paint reagents for antibody screening run or with cell paint reagents only for cell culture optimization run. See *Sample Preparation* on page 10, *Teton Custom Antibody Screening* on page 15, and *Teton Cell Culture Optimization* on page 22.

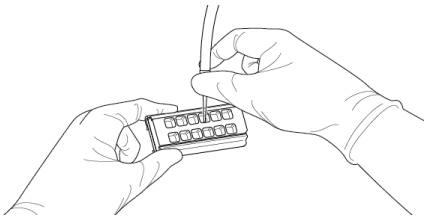
Assemble the Teton Flow Cell

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 flow cell sealer
- Teton flow cell assembly kit

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.

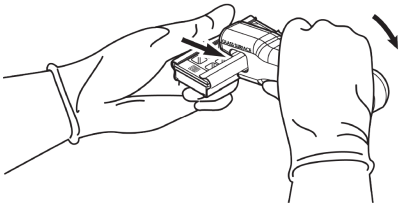


> Perform step **2** to remove the metal side clips using the Teton slide kit tool (part # 810-00021).
> Otherwise, perform step **3**.

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.



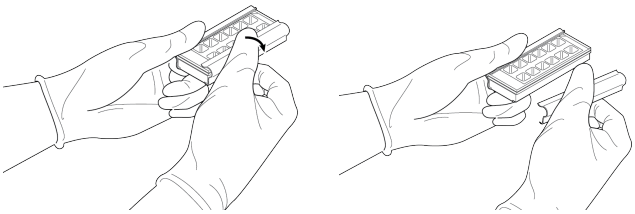
d. Repeat step 2 to remove the metal side clip from the opposite side of the slide kit.

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

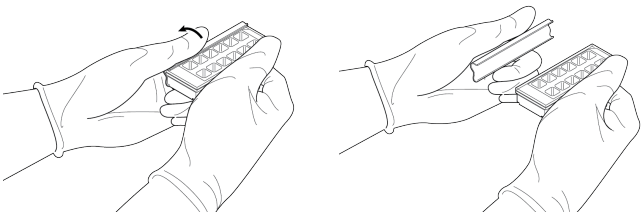
a. Turn the slide kit upside down so the open wells are facing downward and the glass slide is facing upward.

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.

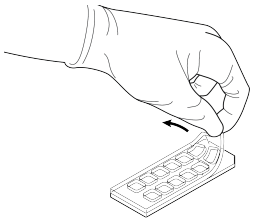


c. To release the second clip, place your thumb on the top-center location of the side clip, and rotate the top edge of the side clip outward with smooth and consistent movement.



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.

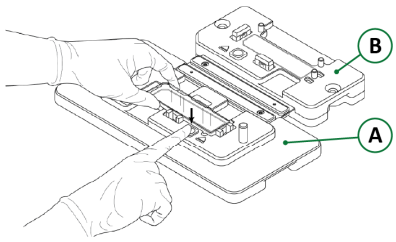


Align and Seal the Slides

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

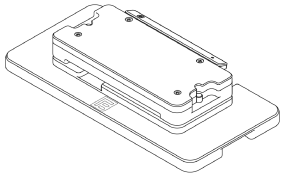
CAUTION
Avoid damage to the slide. Handle slides with care to avoid breakage. Follow instructions to avoid chipping of the edges.

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.

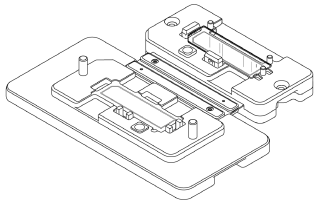


A **Sample** side
B **Adhesive** side

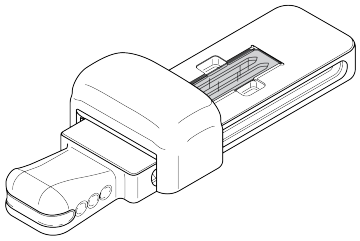
3. Open the Teton Flow Cell Assembly Kit and remove the adhesive slide from the package. Handle the slide from the edges only and make sure the slide is free of debris.
4. Load the adhesive slide onto the flow cell aligner:
    a. Press and hold the button on the **Adhesive** side of the flow cell aligner.
    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.
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.

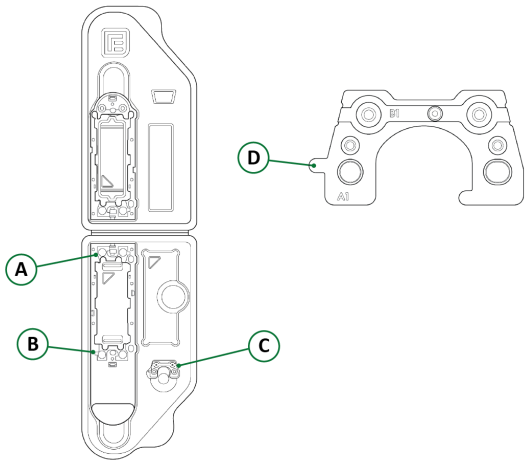
    NOTE
    Moving the roller grip slowly ensures a proper seal and avoids damage to the slides.

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 Cartridge

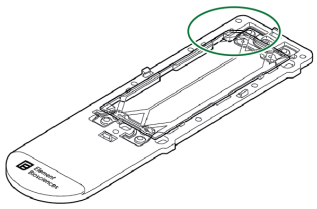
1. Position each of the two flow cell gaskets onto the bottom half of the flow cell cartridge, one above and one below the slide area. Make sure the gasket key is properly seated in the recess.

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



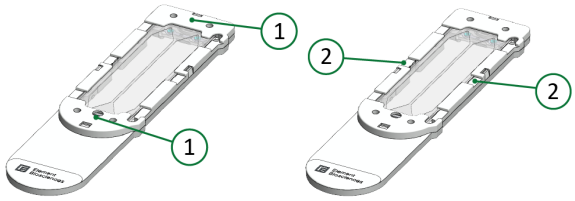
A Gasket location above slide area
B Gasket location below slide area
C Gaskets stored in flow cell assembly package
D Gasket key

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



4. Align the top half of the flow cell cartridge over the bottom half.

5. 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.



6. Visually inspect the flow cell cartridge to make sure there are no gaps along the sides of the cartridge top and bottom. If a gap is visible, repeat step **5** to ensure the cartridge top and bottom 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 *Set Up a Custom Screen Cartridge Run* on page 33.

Set Up a Custom Screen Cartridge Run

1. If applicable, stage run manifests for import:
   - » If setting up the run manually, save the manifest on a USB and connect the USB drive to an instrument USB port.
   - » Alternatively, you can save the manifest to the specified SMB storage connection.
   - » If you planned the run in Elembio™ Cloud, upload the manifest to the planned run.
2. On the Home screen, select **New Run**.
3. For run type, select **Cytoprofiling**.
4. Select a side or both sides to use for the run.
   - » **Side A**—Set up a run on side A.
   - » **Both**—Set up antibody screening run or cell culture optimization run on side A and a full Teton protein run on side B.
   - » **Side B**—Set up a run on side B.
5. Select **Teton Custom Screen** on the side selected for the run.
6. Select **Next** and proceed to one of the following steps:
   - » For a **Manual Run**, proceed to *Define Manual Run Parameters*.
   - » For a **Planned Run**, proceed to *Select a Planned Run*.

Define Manual Run Parameters

1. Make sure **Manual Run** is selected for the type of run.
2. In the Run Name field, enter a unique name to identify the run.
3. [Optional] In the Run Manifest field, select **Browse** and import a run manifest.

   You can import a run manifest from an inserted USB drive or from an SMB storage connection.
4. [Optional] In the Description field, enter a description that represents the run.
5. In the Storage drop-down menu, select a storage location or leave the default selection.
6. In the Well Layout field, select **48** or **12**.
7. In the Assay drop-down menu, select the appropriate assay option.
8. For the Expanded Z option, select **Yes** if you are using cells with an increased height or that tend to form dense colonies. Otherwise, select **No**.

   Example cell lines that benefit from the Expanded Z option include Jurkat, MCF-7, and HeLa.
9. Proceed to *Inspect and Mix Reagents* on page 34.

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.

Inspect and Mix Reagents

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

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

Confirm Reagent Preparation

1. Select the **Invert cartridge** checkbox to confirm that reagents are mixed.
2. Select the **Insert into basket** checkbox to confirm that the cartridge is in the cartridge basket.
3. Confirm that the flow cell assembly is complete with no gaps in the sides of the flow cell, and select the **Verify flow cell** checkbox.
4. Select **Next** to proceed to the Load Reagents screen.

Load Cartridge and Buffer

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

Empty Waste and Prime Reagents

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

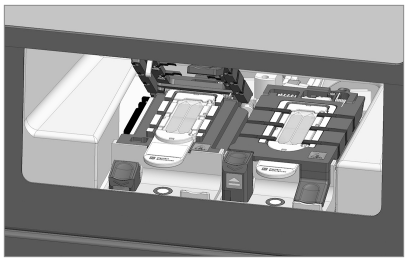
    CAUTION
    Waste bottle contents are considered hazardous. Dispose of waste according to local, state, and regional laws and regulations.
4. Open the transport cap and the vent cap.
5. Support the waste bottle with both hands and empty the waste:
    a. Position the bottle over the funnel or waste receptacle.
    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 10 minutes.
9. When priming is complete, select **Next** to proceed to the Load Flow Cell screen.
   AVITI OS moves the nest forward and opens the nest bay door. A brief delay is normal.

Load the Flow Cell

1. Make sure the nest status light is blue.
2. 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.—
3. Remove the used flow cell from the nest.
4. With the label facing up, place the assembled Teton flow cell over the three registration pins on the nest.



5. Lower the tab on the right side of the lid until the lid snaps into place.
   —The nest status light turns green.—
6. Select **Close Nest** to close the nest bay door and retract the stage.
7. Select **Next** to *automatically* start the Flow Cell Integrity Test.
8. After the Flow Cell Integrity test successfully completes, select **Next**.

Review and Start the Run

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

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

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

Field	Description
Lot Number	The manufacturing batch number assigned to the consumable
Expires on	The date that the cartridge and buffer bottle expires

Field	Description
Serial Number	The unique identifier for the consumable or all zeros indicating an unscanned barcode
Part Number	The part identifier for the consumable

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

3. Select **Run** to start the run.

   The antibody screening run takes approximately 4 hours to complete while the cell culture optimization run takes 3 hours to complete.

Monitor Run and Metrics

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

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

   Following metrics appear as charts depending on the assay selected during the antibody screening run:

   » Quality Control: Shows counts per cell for each well of the positive and negative control included in the Teton Custom Antibody Screen Control. Well number shown in red indicates an error.
   » Thumbnails: Shows cell sample quality, morphology, and confluency.
   » Custom Antibody Count: Shows count per cell along with well location and custom antibody name if provided in the run manifest.

Assay	Metric Charts
Cell Paint + Control Antibodies + Custom Antibodies	• Thumbnails • Quality Control • Custom Antibody Count
Cell Paint + Control Antibodies	• Thumbnails • Quality Control
Cell Paint Only	• Thumbnails

   Additionally, Cell Count, Cell Confluency, and an average across all wells for Positive and Negative controls are also displayed as single metrics (not in charts).

3. When the run is complete, leave all materials on the instrument.

   » To return to the Details view, select **Overview**.
   » To access run data, go to your storage location.

Run Results

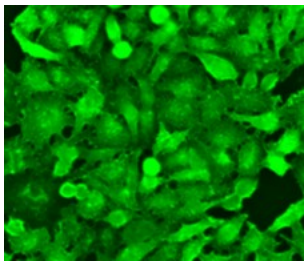
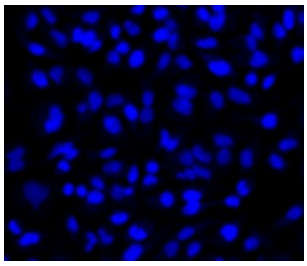
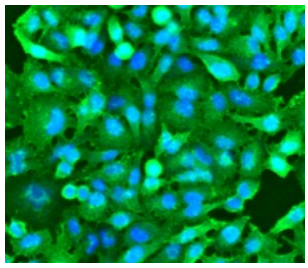
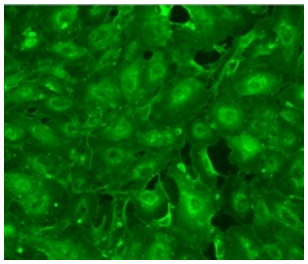
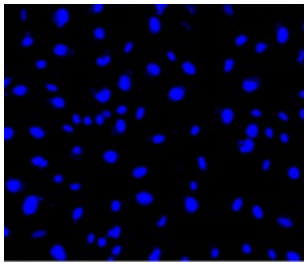
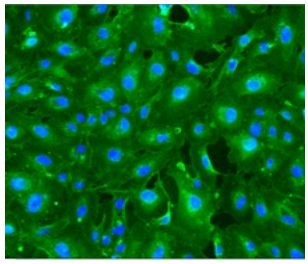
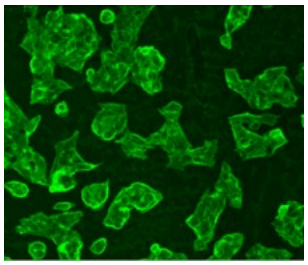
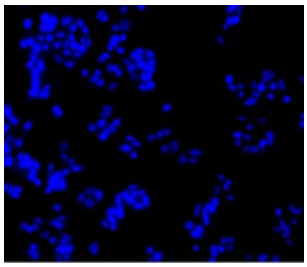
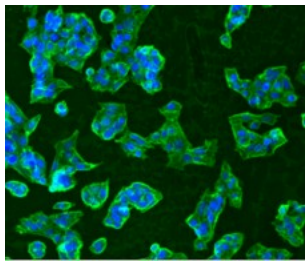
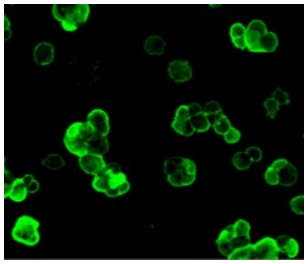
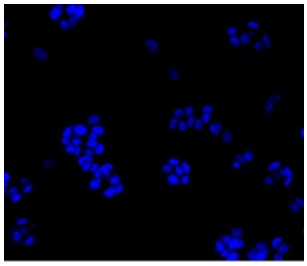
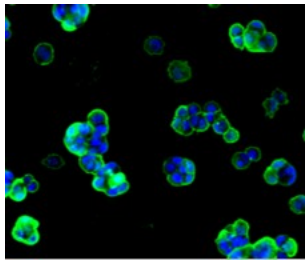
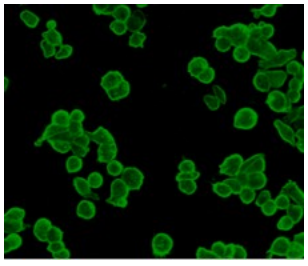
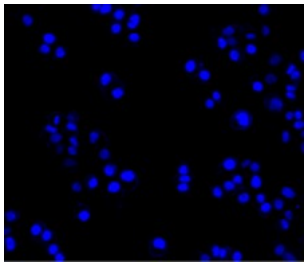
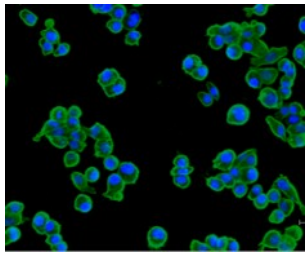
For an antibody screening run:

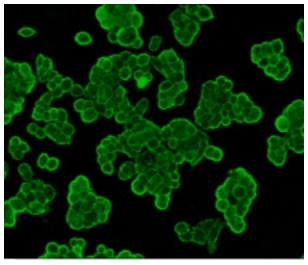
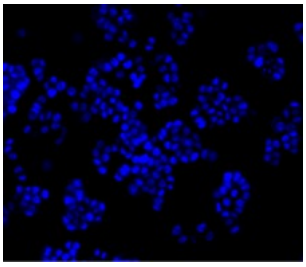
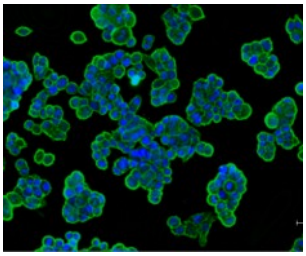
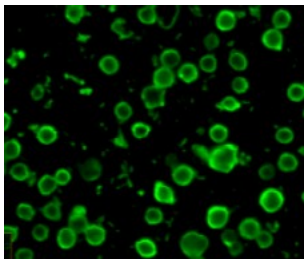
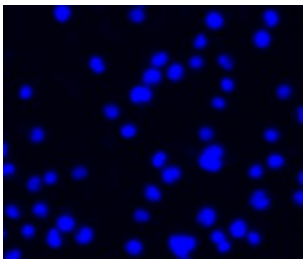
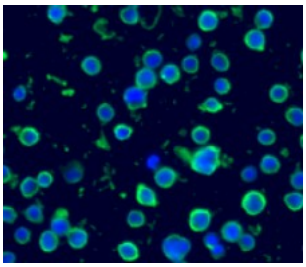
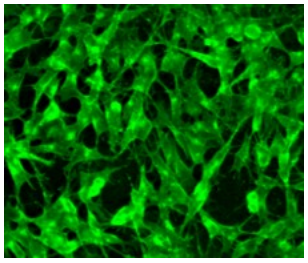
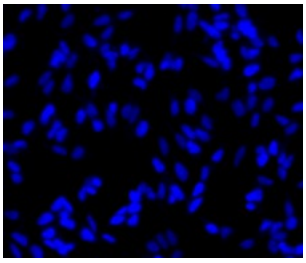
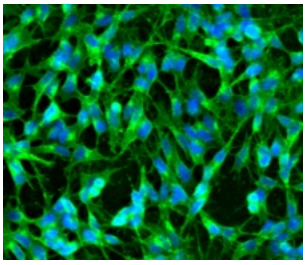
- Review the target counts/negative control counts per cell (normalized values) in the AntibodyScreenKitReport.csv file to ensure these values are > 4 for adherent cells and > 8 for suspension cells, then the custom primary antibody is considered PASSED for detection. This report also includes cell count, cell diameter, and cell area data.
  If you performed the assay without custom primary antibodies (Cell Paint + Control Antibodies), ensure the positive control counts per cell are > 1 to confirm that the assay worked.
- Assess proper segmentation and custom primary antibody localization using CytoCanvas™ to confirm that localization is as expected and does not demonstrate a huge deviation from expected localization.
- Review RawCellStats.csv file for CellProfiler metrics.

For a cell culture optimization run:

- Assess run results using CytoCanvas to inspect cell culture quality.
- Review the AntibodyScreenKitReport.csv for cell count, cell diameter, and cell area data.
- Review RawCellStats.csv file for CellProfiler metrics.

The following examples show successful cell culture technique, confluency, morphology, and localization for some commonly used cell lines using the Teton Onboard Cell Paint Imaging Kit and imaged on an AVITI24 System.

Cell Type	Cell Membrane	Nucleus	Merged
HeLa			
HUVEC			
HEPG2			
MCF7			
PC3			

Cell Type	Cell Membrane	Nucleus	Merged
HCT116			
Jurkat			
SH-SY5Y			

CHAPTER 6

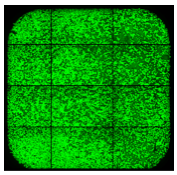
Troubleshooting

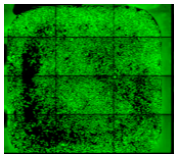
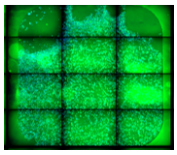
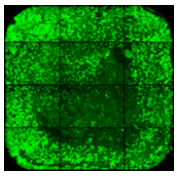
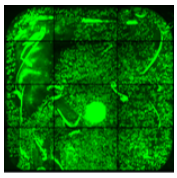
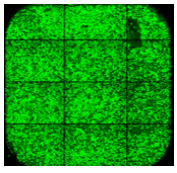
Review the information in this section to troubleshoot issues with cell culture optimization or antibody screening.

If a problem persists, contact Element Technical Support.

Troubleshoot Sample Preparation

The following images show results of successful and unsuccessful sample preparation.

Successful Result		Recommendation
Successful preparation technique		To ensure best results, practice pipetting techniques, such as slow pipetting speed. Avoid creating bubbles or scratching the surface. Follow protocol guidelines for slide kit and pipette position as described in *Protocol Guidelines* on page 10.

Unsuccessful Result		Recommendation
Ring pattern		Avoid circular movement of the pipette or the slide kit when dispensing cells. Use a side-to-side movement for a uniform cell distribution.
Cell loss in the corners of the well		Pipette slowly to prevent bubbles when dispensing cells. Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.
Cell loss in the middle of the well		Pipette slowly to reduce liquid impact on the slide surface after cell attachment.
Scratch marks on the surface		Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.
Cell loss in random locations		Avoid pipette tip contact with the surface of the slide during protocols, such as surface coating preparation and cell culture.

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

Problem	Resolution
Cells are not properly adhered to the slide	Review the surface coating protocol and ensure incubator settings are as recommended for cell line growth conditions.
Cell confluency is below desired amount	Perform a titration to increase seeding density per well for the cell line.
Cell density is higher on the edges than in the center of the well	Distribute cells by sliding the slide kit back and forth gently as described in the seeding portion of the user guide. See *Culture Adherent Cells* on page 11.
Clear indicators of poor fixation such as cell loss/lifting, patches of missing cells, scratched sections of cells, or regions of low confluency/donut-ring shaped low confluency	Review the fixation protocols in this user guide and execute caution when fixing to prevent cells from drying out. Do not fix more than three slides at a time for fixation.
Probes are not producing sufficient intensity for the flow cell (applicable to only Teton Cell Paint Probe Kit)	If imaging on a fluorescent microscope: • Confirm the laser wavelength for excitation and probe emission for the fluorescent microscope • LED light sources might not be as effective at producing intensity of probes • Modify laser power and exposure times to obtain desired intensity, targeted 30 mW power output can vary depending on the instrumentation used If imaging on AVITI24 shows low intensity, increase incubation time for the Teton Cell Paint Probe Kit by 20 minutes.

Troubleshoot Antibody Screening Results

The following troubleshooting information addresses problems that can occur during an antibody screening run setup using the Teton Custom Antibody Screening Kit.

Problem	Resolution
Target counts/negative control counts per cell (normalized values) are 1–4 for adherent cells or 1–8 for suspension cells	Repeat antibody screening run with this primary antibody for another replicate to confirm compatibility. If counts are still low, proceed with testing an alternative custom primary antibody.
Target counts/negative control counts per cell (normalized values) are ≤ 1, indicating that the custom primary antibody is considered FAILED for detection	Ensure there was no error during the custom primary antibody probe formation step. Repeat antibody screening run with this antibody for another replicate or proceed with testing an alternative custom primary antibody.
Positive control shows fewer counts than negative control	Review and follow the antibody dilution and incubation protocol. See *Prepare Antibody Pool and Cell Samples* on page 17.
Negative control counts (> 5) are above the positive control indicative of non-specific binding	Ensure the fixation and wash steps were performed as described in this guide.
Well number is shown in red indicating an error	View the thumbnails to ensure that there are cells in the well. If there are no cells, review sample preparation guidelines and fixation protocol in this guide. To ensure cells are seeded evenly across flowcell, resuspend cells occasionally.
Majority of cells are not segmented indicating cell segmentation failure	• Review the Thumbnails panel to view cell paint images. • Ensure the cell paint incubation was performed for 5 minutes. See *Add Teton Cell Paint Reagents* on page 21. • Certain cell types require longer incubation for up to 20 minutes with Teton Cell Paint Probe Kit to improve the signal and cell segmentation. Optimize incubation time with Teton cell paint reagents using a time titration for the cell line. • Perform resegmentation as using a custom model or an appropriate model for the specific cell type. See Resegmentation Tutorial.
Too many polonies are generated and > 25% of the Cell Channels signal on CytoCanvas is not assigned to the target	Perform the following adjustments on CytoCanvas in the Imaging Panel: • Turn **ON** Cell Boundaries and turn **OFF** all other Cell Channels and Nuclear Boundaries • Under Imaging/Cell channels, turn on **Custom_Protein_1 or Custom_Protein_2** • Turn on the assigned counts for the Protein/custom antibody of interest. Check overlap of assigned and Custom_Protein_1 or Custom_ Protein_2 signal to confirm sufficient assignment of polonies. Perform another antibody screening run with titration of the target to identify an acceptable custom antibody concentration for a Teton run.
Custom primary antibody protein counts are very low or below the range of sensitivity for regulation	Increase custom primary antibody counts by using the same primary antibody as protein 1 and protein 2 in the same well instead of using two different primary antibodies in the same well.

CHAPTER 7

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 Screening and Assessment Kits

Part Number and Kit Name		Quantity	Shipping	Storage
860-00035	Teton Custom Antibody Screening Kit [1]			
	Teton Custom Screen Cartridge (820-00037)	1	-25°C to -15°C	-25°C to -15°C
	Teton Custom Antibody Screen Probe Kit (830-00035) • Teton Custom Antibody Screen Control • Teton Custom Antibody Screen Protein 1 • Teton Custom Antibody Screen Protein 2 • Teton Custom Antibody Screen Dilution Buffer	1	-25°C to -15°C	-25°C to -15°C
	Teton Cell Paint Probe Kit (830-00045) • Teton Optimization Cell Paint Probes • Teton Optimization Wash	1	-25°C to -15°C	-25°C to -15°C
	AVITI Buffer Bottle (Universal Wash Buffer) (820-00002)	1	Room temperature	Room temperature

[1] For screening antibody selections before using the Teton Custom Add-On Protein Panel Assembly Kit. See *Teton Custom Antibody Screening* on page 15.

Part Number and Kit Name		Quantity	Shipping	Storage
830-00045	Teton Cell Paint Probe Kit [2] • Teton Optimization Cell Paint Probes • Teton Optimization Wash	1	-25°C to -15°C	-25°C to -15°C

[2] For assessing sample preparation results on a Teton slide kit. See *Teton Cell Culture Optimization* on page 22.

Part Number and Kit Name		Quantity	Shipping	Storage
860-00047	Teton Onboard Cell Paint Imaging Kit [2]			
	Teton Custom Screen Cartridge (820-00037)	1	-25°C to -15°C	-25°C to -15°C
	Teton Cell Paint Probe Kit (830-00045) • Teton Optimization Cell Paint Probes • Teton Optimization Wash	1	-25°C to -15°C	-25°C to -15°C
	AVITI Buffer Bottle (Universal Wash Buffer) (820-00002)	1	Room temperature	Room temperature

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

Part Number and Kit Name		Quantity	Shipping	Storage
860-00031	Teton Slide Kit, PLL – 12 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

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
Cover slips	General supplier
Dulbecco's Phosphate Buffered Saline (DPBS), 1X, pH 7–7.4	Gibco, 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	ThermoFisher Scientific, catalog # TS 4662090 General lab supplier
Reagent trough	General lab supplier
RiboLock RNase Inhibitor	ThermoFisher Scientific, catalog # EO0381
For use with antibody screening protocol: 96-well plates 0.5 ml low-bind tubes 2 ml low-bind tubes	General lab supplier

Document History

Revision	Description of Change
July 2025 Document # MA-00078 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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