

Teton™ Cell Culture Optimization

Guidance for optimizing cell culture and imaging for Teton CytoProfiling

Highlights

- Assess up to 48 cell culture conditions or cell lines
- Select from flexible kit options
- Obtain results in 1 or 3 hours

Introduction

Proper cell culture techniques and cellular morphology are key to a successful Teton CytoProfiling run.

The Teton Cell Paint Probe Kit and Teton Onboard Cell Paint Imaging Kit when used in conjunction with bright field microscopy offer rapid and flexible options with improved resolution for evaluating critical cell culture variables within 1 or 3 hours, respectively. Both kits allow you to assess a variety of cell lines or culture conditions such as cell morphology, cell growth, and compatibility with the Teton platform using a 48-well or 12-well Teton Slide Kit. This high-throughput capability allows for rigorous cell culture optimization, ensuring robust performance in downstream Teton CytoProfiling runs.

The Teton Cell Paint Probe Kit protocol prepares the sample slide for viewing under a fluorescent microscope providing clear visualization of cell morphology and cell quality. The Teton Cell Paint Probe Kit includes the Teton Optimization Cell Paint Probes for cellular visualization and a Teton Optimization Wash buffer.

The Teton Onboard Cell Paint Imaging Kit protocol prepares the sample slide for imaging on an AVITI24™ System, which offers a comprehensive cellular readout along with cell morphology, cell confluency, and CellProfiler values. The Teton Onboard Cell Paint Imaging Kit includes the Teton Custom Screen Cartridge, Teton Cell Paint Probe Kit, and AVITI™ Buffer that allows for one cell culture optimization run on an AVITI24 System.

Workflow

Prior to a Teton CytoProfiling run, optimize cell culture on 96-well or 384-well glass plates with a desired surface coating, and assess cell growth and health using bright field microscopy. Using the optimal 96-well or 384-well plate seeding density, perform another cell density titration on a 12-well or 48-well Teton Slide Kit, respectively. Assess cell density on the Teton slide with bright field microscopy to identify ideal seeding density for a Teton assay (Figure 1a). Reference [Cell Culture Hub](#) for cell images and confluency.

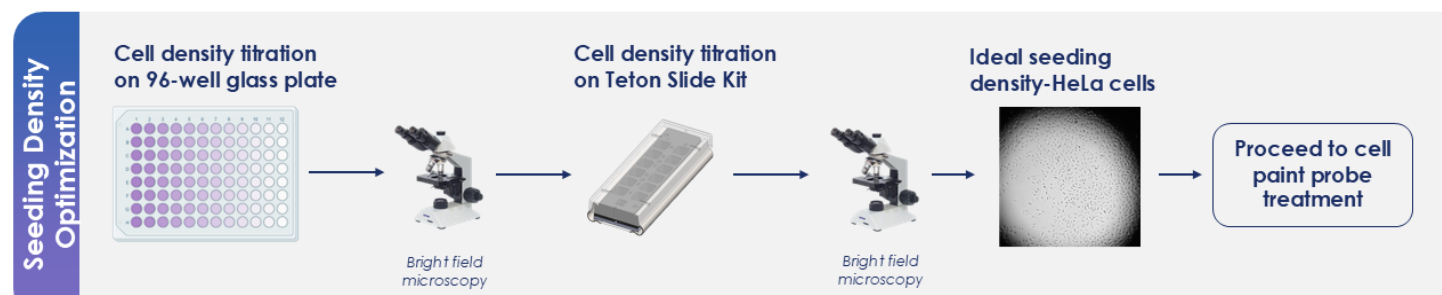


Figure 1a. 96-well glass plate and 12-well Teton Slide Kit seeding density optimization workflow using bright field microscopy.

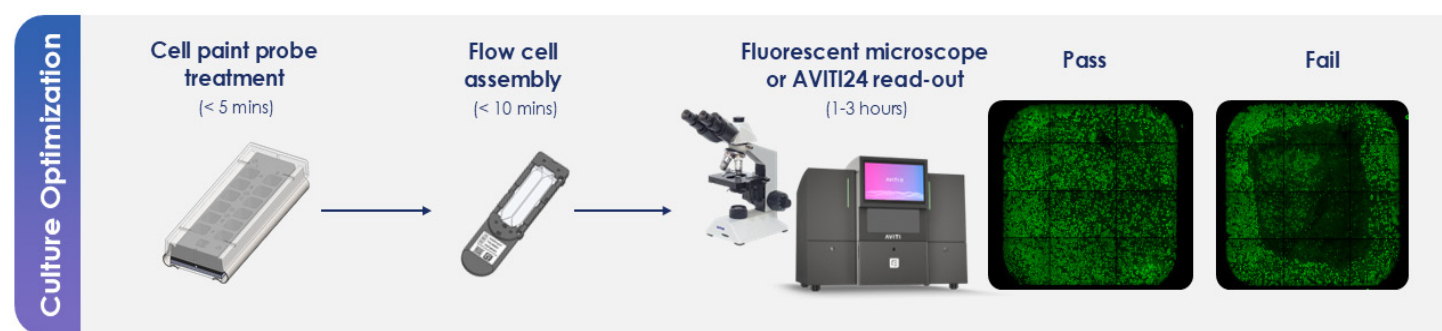


Figure 1b. Sample prep assessment using Teton Onboard Cell Paint Imaging Kit or Teton Cell Paint Probe Kit.

After assessing confluency with bright field microscopy, fix cells on the slide kit using validated protocols to ensure cell adhesion. Incubate samples with Teton cell paint probes and after a wash step, prepare and image the sample slide as follows:

- For Teton Cell Paint Probe Kit, disassemble the slide kit and assemble the sample slide for viewing on a fluorescent microscope (Figure 1b).
- For Teton Onboard Cell Paint Imaging Kit, assemble the Teton flow cell, load the Teton Custom Screen Cartridge and the assembled flow cell onto an AVITI24 System. Initiate the cell culture optimization run and analyze results in 3 hours (Figure 1b).

For protocol details, see the [Teton Optimization and Screening User Guide \(MA-00078\)](#).

Cell Culture Guidance

For optimal results on a Teton run, use adherent cells cultured on a Teton slide at 50-70% confluency for most cells and 30-50% confluency for neurons. Ensure cells are evenly distributed throughout the well, not over confluent, and exhibiting high viability.

For suspension cells, ensure the cells are fully dissociated to form a single cell suspension to minimize cell clumping on the slide and aim for a monolayer confluency of 50-70%.

Optimize seeding density on a glass plate, then titrate cell density on a Teton slide kit to minimize any surface variations and determine ideal seeding confluency for a Teton assay.

The Teton slide kits are compatible with a variety of human cell lines such as adherent and suspension cell lines, immortalized cell lines, and primary cell lines (Table 1). Visit the Element Biosciences [Knowledge Base](#) to view additional cell types that are continuously tested and validated for use on a Teton run.

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

Adherent	Suspension	Primary
HeLa	HL-60	HUVEC
HEPG2	Jurkat	MSC
SH-SY5Y	Raji	PBMC

Table 1. Example list of compatible cell lines for use with Teton slide kit.

Surface Coating

Teton slide kits are available as coated with Poly-L-Lysine (PLL) or uncoated. Surface coating increases cell adhesion to the slide and minimizes cell loss during wash steps. Depending on your specific cell line, you can coat an uncoated glass plate or Teton slide with commonly used surface coating reagents and assess efficacy of these different coating reagents as shown in Table 2.

Select a surface coating that allows proper growth and retains morphology characteristics for your cell line. If there is poor cell adhesion as indicated by cell lifting or peeling, test alternate surface coatings.

Well	1	2
A	Gelatin	Poly L-Lysine (PLL)
B	Laminin	Matrigel
C	Collagen	Fibronectin
D	Fibronectin	Collagen
E	Matrigel	Laminin
F	Poly L-Lysine (PLL)	Gelatin

Table 2. Example of an experimental setup in duplicates for a 12-well Teton Slide Kit or 96-well glass plate for assessing different surface coatings for your specific cell line.

Experimental Setup

As a best practice, always perform the testing in replicates for better statistical significance and data interpretation. Randomizing the sample setup helps in minimizing any performance bias due to well positions on the slide kit or plate. To identify an optimal seeding density for a Teton CytoProfiling run, first perform a cell density titration of your cell line on 96-well or 384-well glass plates. Repeat the titration on a Teton slide kit using the optimal glass plate seeding density. Further confirm the cell adherence and viability using the Teton Cell Paint Probe kit or Teton Onboard Cell Paint Imaging Kit prior to a full Teton CytoProfiling run. Tables 3 and 4 show examples of experimental setup for various sample assessment conditions.

Well	1	2
A	2500 HeLa cells/well	15,000 HeLa cells/well
B	5000 HeLa cells/well	12,500 HeLa cells/well
C	7500 HeLa cells/well	10,000 HeLa cells/well
D	10,000 HeLa cells/well	7500 HeLa cells/well
E	12,500 HeLa cells/well	5000 HeLa cells/well
F	15,000 HeLa cells/well	2500 HeLa cells/well

Table 3. Example of a seeding density experimental setup in duplicates for a 12-well Teton Slide Kit or 96-well glass plate with HeLa cells.

Well	1	2
A	5000 A549	5000 NCI-H1975
B	7000 A549	7000 NCI-H1975
C	9000 A549	9000 NCI-H1975
D	5000 NCI-H1299	5000 PC9
E	10,000 NCI-H1299	7000 PC9
F	15,000 NCI-H1299	9000 PC9

Table 4. Example of an experimental setup for a 12-well Teton Slide Kit or 96-well glass plate with multiple cell lines seeded at different seeding densities. Each well lists the number of cells/well plated for each cell line.

Comparison of Imaging Options

Sample preparation assessment is performed using a bright field microscope followed by a fluorescent microscope using the Teton Cell Paint Probe Kit or on an AVITI24 System using the Teton Onboard Cell Paint Imaging Kit. While the bright field microscope provides a quick and easy option to image your sample slide, the AVITI Operating Software provides rich and actionable onboard data output in 3 hours along with off-instrument data (Table 5). The onboard data includes quantified cell culture metrics, and images of cell morphology and distribution. The off-instrument qualitative data can be reviewed on Element CytoCanvas™, an easy-to-use, free visualization software.

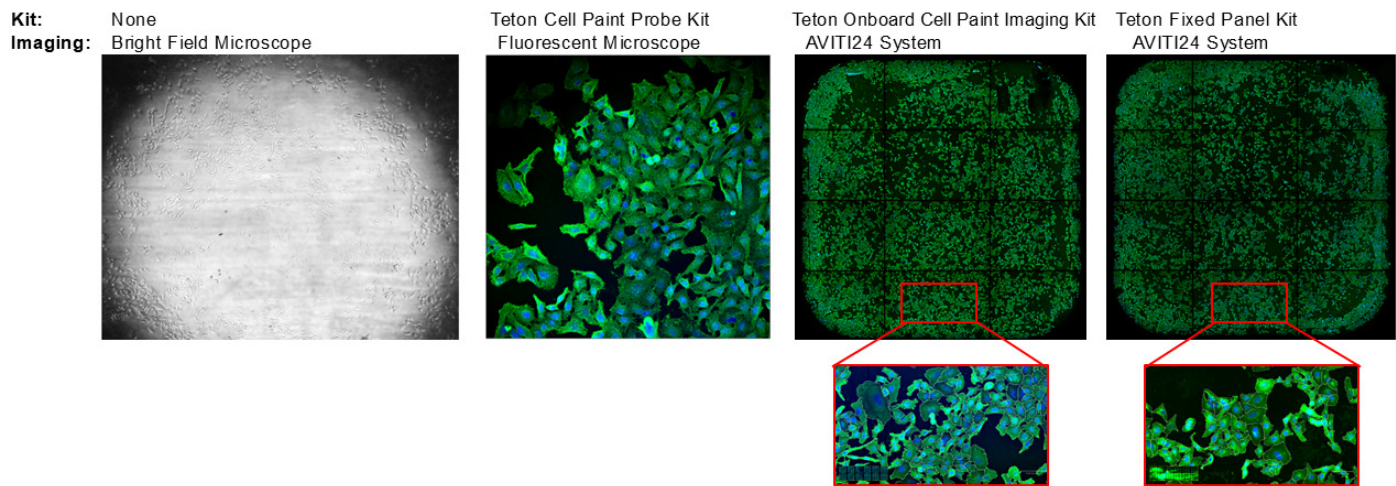


Figure 2. Teton slide kit with HeLa cultured cells imaged using different morphology assessment methods. AVITI24 System images represent the entire flow cell. Inset image shows close up of a subset of the flow cell showing high resolution cell images.

Bright Field Microscope	Fluorescent Microscope	AVITI24 System
Cell morphology	Cell morphology	Cell morphology
—	Cell paint images	Cell paint images
—	—	Cell counts
—	—	Confluency
—	—	Segmentation

Table 5. Data outputs for various imaging options.

A visual comparison of different morphology assessment methods is shown in Figure 2. Bright field microscope allows for fast, low resolution assessment of seeding densities without any details on cell adherence. As compared to a bright field microscope, the fluorescent microscope provides higher resolution of cell morphology, clear definition of cell adherence, and cell culture compatibility with Teton slide kit and cell paint reagents.

Imaging on the AVITI24 System allows visualization of the entire flow cell to easily and accurately assess cell culture confluency. In addition to high resolution imaging on the AVITI24 System, the Teton Onboard Cell Paint Imaging Kit produces rich data output comparable to a Teton CytoProfiling run.

Analyzing Results

Using glass plates and bright field microscopy confirm the optimal seeding density for your specific cell line and surface. (Figure 3). Due to slight adhesion differences between the plate and slide,

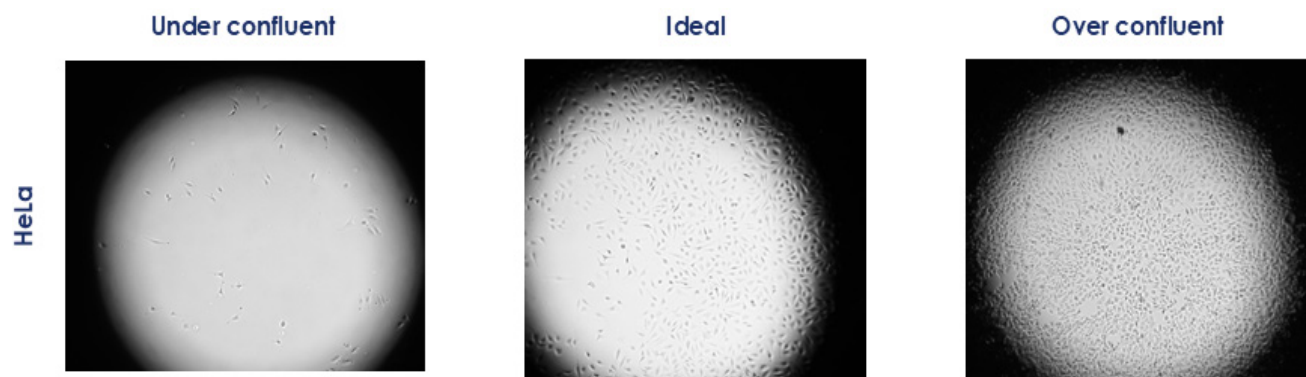


Figure 3. HeLa cells seeded at varying densities on a 96-well glass plate and viewed using an OMAX K832 inverted bright field microscope at 40X magnification to identify optimal seeding confluency. For seeding density titration images of additional cell lines, visit the [Cell Culture Hub](#).

further optimize cell seeding on the Teton slide using bright field microscopy to achieve 30–70% target confluency based on your cell type.

The Teton Cell Paint Probe Kit supplements the initial bright field microscopy results, and provides a qualitative assessment of cell adherence and cell culture techniques using a fluorescent microscope readout. You can use additional microscopy tools to quantify cell count and other cellular metrics. Ensure cells are exhibiting proper morphology with well defined edges and consistent signal distribution across the cell population.

In addition to qualitative data, the Teton Onboard Cell Paint Imaging Kit provides rich, quantitative data including cell count, cell diameter, cell area, and cell segmentation. You can further assess cell culture quality and cell segmentation using CytoCanvas.

Confirm the cell paint signal for cell membrane and nucleus is well above the autofluorescence cell signal.

Figure 4 shows results of a successful cell culture technique for commonly used cell lines using the Teton Onboard Cell Paint Imaging Kit and imaged on an AVITI24 System.

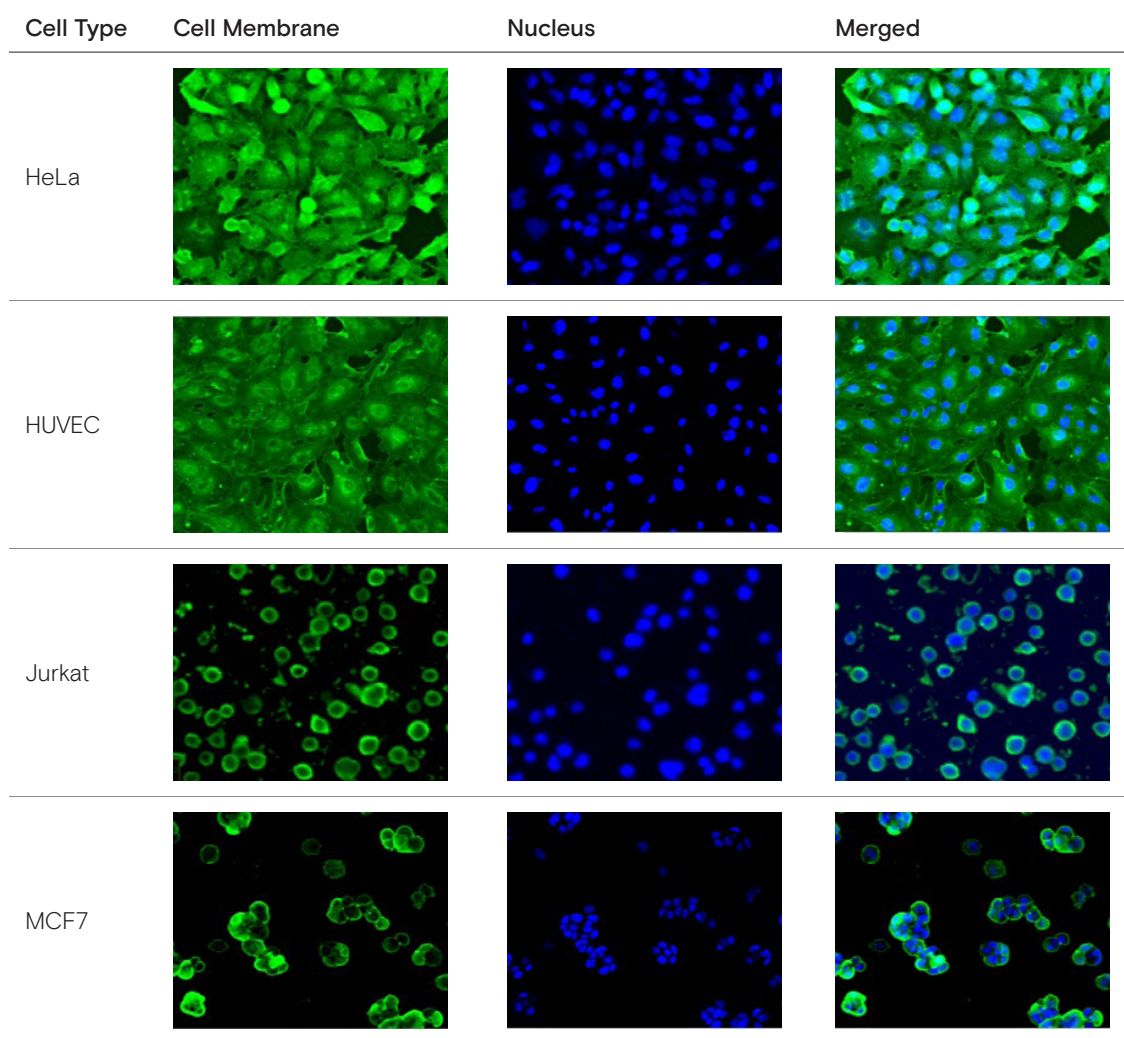


Figure 4. Examples of successful cell culture technique, confluency, morphology, and localization for commonly used cell lines using the Teton Onboard Cell Paint Imaging Kit and imaged on an AVITI24 System.

Summary

Cell culture optimization on a 96-well or 384-well glass plate with bright field microscopy enables rapid assessment of seeding densities, surface coatings, and culture conditions providing an initial compatible cell density range for a Teton assay. The Teton Cell Paint Kit and Teton Onboard Cell Paint Imaging Kit offer distinct cellular insights that further supplement the bright field microscopy results and provide the flexibility you need to fine-tune your cell culture variables prior to a Teton CytoProfiling run.

Both kits generate high resolution cellular readouts in just one

to three hours and offer high-throughput capability allowing you to confidently identify optimal cell culture conditions for a successful downstream Teton CytoProfiling run.

The Teton Onboard Cell Paint Imaging Kit offers additional benefits with comprehensive automated onboard primary data analysis and off-instrument interactive data visualization with CytoCanvas as compared to the Teton Cell Paint Kit.

To learn more, visit elementbiosciences.com

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