

Teton™ Protein Performance on AVITI24™

Balancing plex and sensitivity for reliable single cell protein calls

Highlights

- **Flexible Sensitivity Tuning:** Custom protein add-ons and barcode allocation enable fine-tuned detection of specific or low-abundance targets while maintaining assay robustness.
- **Optimization Framework:** TNF α phospho-signaling studies showed that barcode allocation affects sensitivity and identified a threshold of five barcodes per target, forming the basis for protein optimization on AVITI24.
- **Validated Accuracy:** Protein detection on Teton confirmed by orthogonal and functional assays, demonstrate antibody specificity, biological fidelity, and reproducibility.

Introduction

Teton CytoProfiling on AVITI24 enables simultaneous detection of RNA, protein, phosphoprotein, and morphology at subcellular spatial resolution through a one-day, library-free workflow capable of processing up to one million cells per run. Protein targets are detected using a primary antibody complexed with a non-covalent coordinating molecule linked to a primer and barcode-containing circular DNA molecule (Figure 1) that are subsequently read using standard Avidite Base Chemistry™ (ABC™) sequencing on an AVITI24. A sequencing readout is used to identify a barcode that corresponds to the target protein of interest and correlates to cellular abundance of the target protein. The barcode counts represent a sub-sampling of the total target protein concentration in the cell and can be used to assess differential protein expression between cells within wells or across wells.

When greater sensitivity is required for specific targets for enhanced detection performance, the Teton Custom Add-On Protein Panel Kit offers flexibility in barcode allocation by allowing

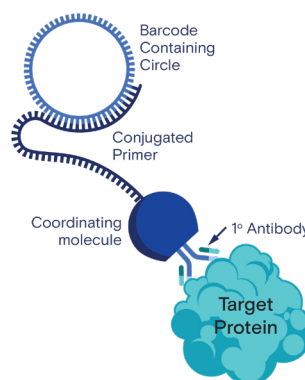


Figure 1. Teton CytoProfiling protein detection schema.

assignment of the same primary antibody to multiple barcodes. Here we describe the validation of protein detection using Teton CytoProfiling on AVITI24 with different methods and a framework for protein optimization using barcode allocation.

Validation of Protein Detection

Protein validation for the Teton CytoProfiling workflow combined immunofluorescence microscopy, Western blot, and functional perturbation assays to confirm antibody specificity and biological accuracy across various cell lines and targets.

Western blotting verified expected molecular weights and confirmed the absence of nonspecific signal (Figure 2). Immunofluorescence microscopy was used as an orthogonal method to confirm antibody reactivity in fixed cells (Figure 2).

Functional validation demonstrated that Teton detection accurately reflects regulated biological changes. TNF α stimulation produced expected phosphorylation events such as phosphorylated HSP27 (phospho-HSP27) which were abolished following p38 inhibitor treatment (Figure 2). siRNA-mediated knockdowns of HSP60, ERK 1/2, MAPK 1, and MAPK3 in HeLa and

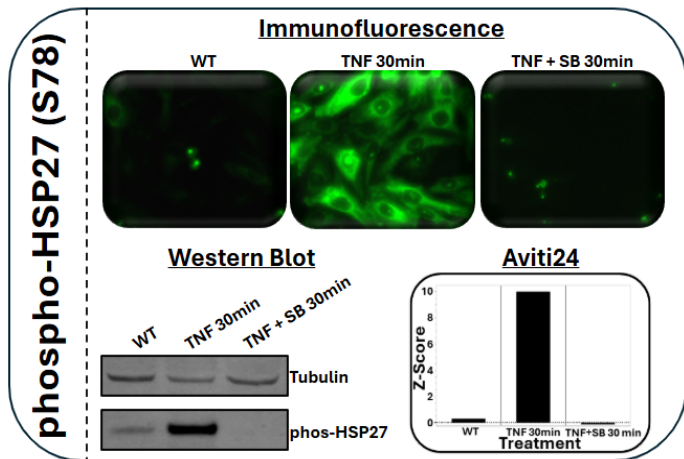


Figure 2. Protein detection validation methods. Phospho-HSP27 levels were measured in HeLa cells following TNF α stimulation for 30 minutes and p38 inhibition with SB202190 (SB). All three methods (AVITI24 bar plot, Western blot image, and immunofluorescence images) showed strong up-regulation of phospho-HSP27 with TNF α treatment and complete suppression upon addition of the p38 inhibitor, SB.

A549 cells further confirmed that reduced expression corresponded with decreased Teton-derived signal (Figure 3). To assess run-to-run reproducibility and phosphoprotein specificity, sixteen replicate runs across four AVITI24 instruments were performed using the full Teton MAPK-Apoptosis panel, with untreated HeLa cells in one lane and Lambda Protein Phosphatase (LPP)-treated HeLa cells in the other lane of a 12-well Teton Slide Kit.

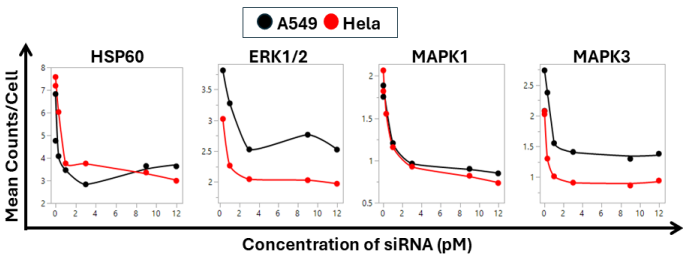


Figure 3. Dose-response profiles for HSP60, ERK 1/2, MAPK 1, and MAPK3 across increasing siRNA concentrations in A549 (black) and HeLa (red) cells. Each plot shows mean counts per cell demonstrating robust, saturating knockdown effects.

Reproducibility was high (mean pairwise $R^2 = 0.975$ across runs, $R^2 > 0.98$ between replicate wells). Phospho-T197-PKA showed a -60.1% mean signal reduction with 4.7% standard deviation following LPP treatment, demonstrating the sensitivity and robustness of Teton CytoProfiling assay for detecting phosphorylation state. Together, these orthogonal and functional validation experiments confirm that Teton CytoProfiling assay on AVITI24 produces accurate, biologically consistent detection of protein and phosphoprotein across multiple targets and cell systems¹.

Protein Configuration and Customization Options

Teton panels provide configurable options to meet variable experimental needs and include Teton Fixed Panels, mix-and-match Teton Focus Protein Panels, and Teton Custom Protein Add-On Panel Assembly Kit (Table 1). The protein panel compatibility differs and is based on the type of Teton cartridge (Table 2).

Features	Teton Fixed Panels	Teton Focus Protein Panels	Custom Protein
Plex	50-plex	24-plex	Up to 88-plex
Description	Pre-designed, curated set of fixed proteins targeting specific biological processes. Choose from three 50-plex protein panels that are available as standalone (Teton Atlas High Output) or in conjunction with 350-plex RNA targets (Teton).	Nine mini, pre-designed, 24-plex protein panels targeting focused research areas. Mix and match up to 3 focus panels or combine 1-2 focus panels with up to 24 custom antibodies. Select only one focus panel from each set at a time.	Use the Teton Custom Add-On Protein Panel Assembly Kit to build up to 88 additional protein targets of your choice.
Content	- Teton Human Neuro Panel - Teton Human Immuno Panel - Teton MAPK-Cell Cycle-Apoptosis Protein Panel	- Set 1: Cytokine Signaling, Cell Metabolism, Neurodegeneration - Set 2: T Cell Activation, Cell Stress & Apoptosis, Neurodevelopment - Set 3: Innate Immunity, Gene Regulation, Neuroinflammation	Custom Protein

Table 1. Protein panel options available for Teton CytoProfiling.

Cartridge	Teton	Teton Atlas™ Low Output	Teton Atlas High Output <i>(Coming soon!)</i>
Core technology	Multiplexed targeted RNA & protein panels with cell paint	Direct In Sample Sequencing (DISS) for native guide RNA sequencing, targeted protein, and morphology by cell paint.	Direct In Sample Sequencing (DISS) for native guide RNA sequencing, 3' transcriptome, targeted protein, and morphology by cell paint. Available in various configurations.
Range	50–138 targets	1–88 targets*	0–88 targets**
Teton Fixed Panels	✓ Included in Teton Panel Kit	✗	✓ Add-on Option
Teton Focus Protein Panels	✓ Add-on Option	✓ Add-on Option	✓ Add-on Option
Custom Protein	✓ Add-on Option	✓ Add-on Option	✓ Add-on Option

Table 2. Teton cartridge types and protein panel compatibility.

* Ensure there are a minimum of 24 barcodes for the assay. If you are using only a single add-on target, add the same primary antibody to 24-wells of the custom protein plate to achieve 24 barcodes required for the assay.

** Some High Output configurations do not include protein to enable more detection of poly-A transcripts.

Teton Custom Protein Add-On Panel Assembly Kit

The Teton Custom Protein Add-On Panel Assembly Kit includes reagents to couple up to 88 user-supplied primary antibodies to Teton detection probes without conjugation steps. The kit contains a plate with individual wells that contain coordinating molecules conjugated to a primer and barcode-containing circular DNA which are incubated with up to 88 custom, rabbit-derived primary antibodies in a plate-based format. After a 1-hour incubation, each custom antibody is now coupled to a unique barcode. To increase sensitivity for a specific target, use the same primary antibody in multiple wells to increase total probe concentration in a 1:1 ratio. This enables the counts to be distributed across multiple barcodes, preserving color diversity during barcode sequencing. Thus, the kit provides a way to increase sensitivity for selected targets and enables the assembly of Teton probes at varying sensitivities.

The Teton Custom Antibody Screening Kit offers an easy workflow to validate custom antibodies prior to running a full Teton CytoProfiling custom workflow as antibody performance can vary by clone or vendor. Refer to the [Teton Custom Antibody Screening Tech Note](#) and [Teton Optimization and Screening User Guide](#) for protocols to test multiple antibody batches and clones across different surfaces and seeding densities. The resulting quantitative metrics (counts/mm²) and visual inspection help identify high-performing candidates, and guide decisions on barcode allocation and antibody concentrations.

Optimization of Barcode Allocation

When higher sensitivity is required for certain targets, the Teton Custom Add-On Protein Panel Kit workflow allows the same primary antibody to be assigned to multiple barcodes to improve detection performance.

The subsequent section uses TNFα stimulation and phospho-HSP27 as a controlled model to characterize detection limits on AVITI24 and illustrate that barcode allocation strategies influence sensitivity.

Model System and Experimental Setup

HeLa cells were stimulated with TNFα to generate a defined phosphorylated signal in HSP27, which is known to activate MAPK and ERK survival pathways to promote anti-apoptotic activity.

To vary response magnitude, cells were treated with TNFα for 5 minutes (subtle response) or 30 minutes (strong response). Stimulated cells represented the positive condition, while unstimulated cells served as negative controls. Experiments were performed using the Teton Human MAPK–Apoptosis Panel Kit (which includes phospho-HSP27) and 12-well, PLL-coated Teton Slide Kit.

 = Barcode assigned to phospho-HSP27

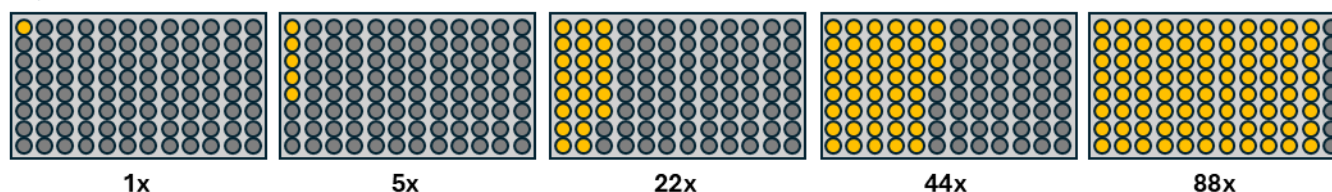


Figure 4. Phospho-HSP27 barcode assignment schema using the Teton Custom Protein Add-On Panel Assembly Kit that includes the plate with 88 wells containing coordinating molecules which bind to custom, rabbit-derived primary antibodies and allows customization of protein targets.

To assess the effect of increasing barcode allocation on detection sensitivity and potential saturation effects, 1, 5, 22, 44, and 88 custom protein barcodes were allocated to anti phospho-HSP27 primary antibody using the Teton Custom Add-On Protein Panel Assembly Kit (Figure 4). Each barcode allocation level was then run as a full Teton CytoProfiling run on AVITI24, resulting in five total Teton runs to generate the barcode allocation data. A Teton Diversity Spike-In was included if less than 24 protein targets were present in the panel.

Detection sensitivity was quantified using mean counts per cell and Power (N). Power is defined as the bootstrap probability that the mean of the positive condition exceeds the 95th percentile of the negative condition at a given sample size N. In other words, Power is the ability to discern between 2 conditions at a given sample size. Power (1) represents single-cell sensitivity.

Quantitative Assessment of Sensitivity and Power

Mean counts per cell scaled approximately linearly with barcode allocation (Figure 5). Subtle TNF α responses (5 minutes) produced lower mean counts/cell than strong responses (30 minutes) at all barcode allocations.

Power increased with effect magnitude and sample size, and provided a more interpretable measure of true detection sensitivity (Figure 6). Strong TNF α responses correspond with pronounced molecular and phenotypic changes compared to subtle responses, enabling greater Power at all sample sizes. Power consistently increased as more cells were sampled. Across both conditions, Power reached an inflection point near five barcodes per target, where additional allocation offered minimal sensitivity gain but increased nonspecific signal (Figure 6). This can be observed visually as excessive barcode allocation reduced specificity due to nonspecific binding (Figure 7).

The following section reviews final barcode allocation choices in the context of experimental goals, target protein concentration, and data interpretation alongside barcode concentration and total signal density.

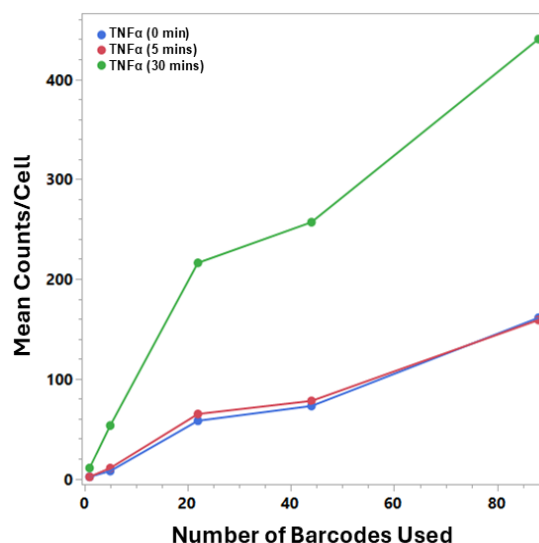


Figure 5. Mean counts per cell increases linearly with barcode assignment. Prolonged TNF α treatment (30 minutes) produced significantly more mean counts/cell than subtle (5 minutes) or no TNF α treatment.

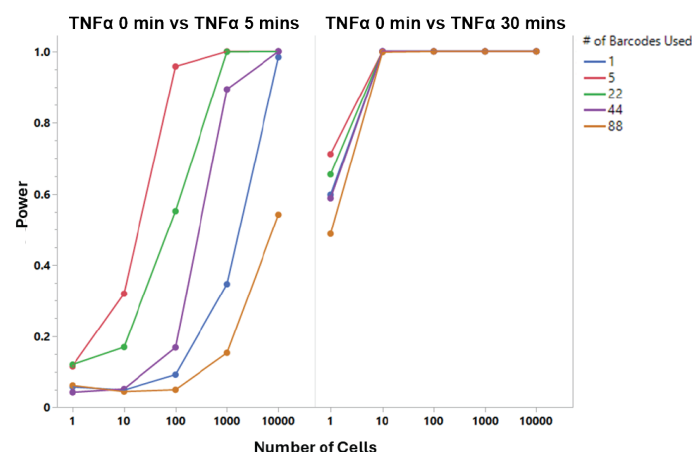


Figure 6. Power vs barcode allocation curves for phospho-HSP27 for HeLa cells treated with TNF α for zero and 5 minutes (left), and zero and 30 minutes (right).

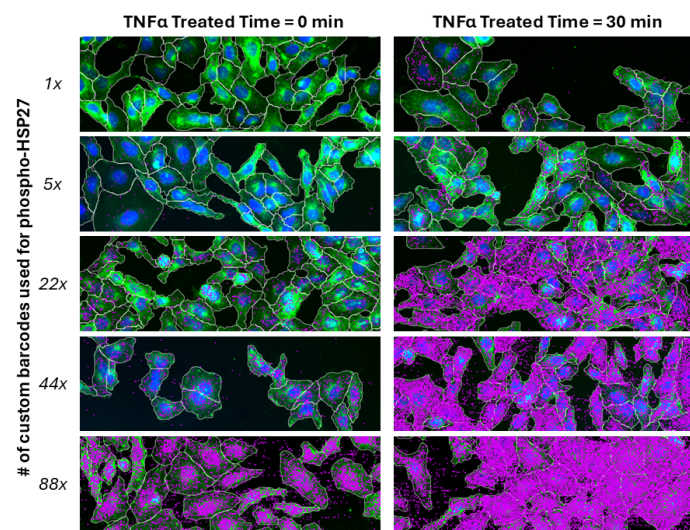


Figure 7. Signal saturation of phospho-HSP27 (shown in purple) as barcode allocation increases for TNF α stimulation from zero to 30 minutes.

Barcode Concentration and Practical Allocation Limits

In Teton protein workflows, barcodes are used at empirically selected working concentrations within an optimal allocation range. Teton Focus Panels and Teton Custom Protein Add-On Panel barcodes are run at 1X concentration while the Teton Fixed Panels are run at 3–5X (Teton Human MAPK–Cell Cycle Panel at 3X, Teton Neuro and Immuno panels at 5X). Teton Focus Panels and the Teton Custom Protein Add-On Panel have lower barcode concentration to enable you to balance plexity and sensitivity in a tunable fashion tailored to specific experimental goals. These settings reflect allocation levels that allow reliable target detection without nearing saturation and are consistent with the empirical guideline of using about five barcodes per target as a starting point to balance sensitivity with barcode usage.

Optimal barcode allocation for a given target varies with antibody dissociation rates, target expression, and specific experimental conditions (Figure 8). The Teton Custom Antibody Screening Kit, recommended for any assay using a custom antibody, helps refine allocations by reporting counts per mm² for every antibody tested as an estimate of signal density. A single batch saturates at roughly 500 K/mm², but a conservative upper limit of 400 K/mm² is recommended. The total counts across all custom antibodies in a batch should remain below 400 K/mm².

Because counts scale approximately linearly with allocation, use the screening results to project the effect of different barcode allocation schema. For example, if three custom proteins yield 50 K/mm², 10 K/mm², and 25 K/mm² per barcode from the Teton Custom Antibody Screening Kit, various distributions can meet the 400 K/mm² limit when deciding on barcode allocation for the

full Teton run. One option is to assign eight barcodes to the first protein only, which projects to 400 K/mm². Another option is to distribute barcodes across all three proteins, such as four to the first, ten to the second, and four to the third, which also projects to 400 K/mm². Generally, proteins with lower count density tend to benefit more from increased barcode allocation than those with higher count density as they typically have proportionally less nonspecific binding.

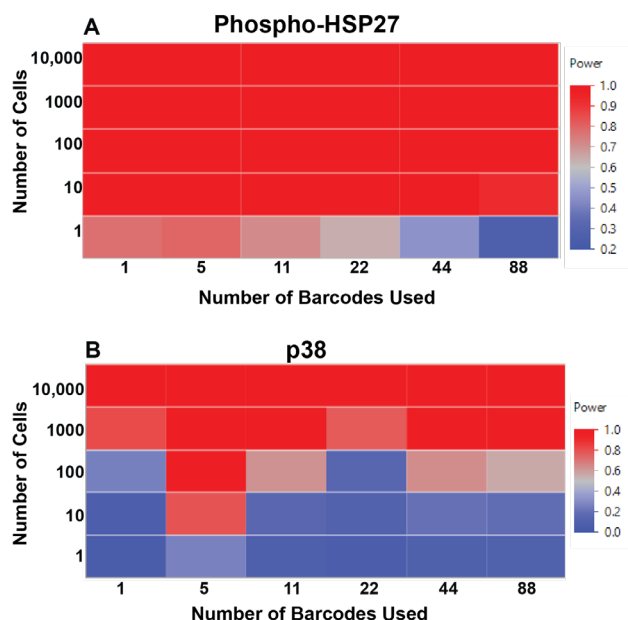


Figure 8. Power metrics by number of cells and barcode allocation for phospho-HSP27 (A) and p38 (B) in HeLa cells treated with TNF α for zero and 30 minutes show power differences for 2 separate targets in the same run.

This allows you to prioritize sensitivity while keeping total density below the recommended cutoff.

This framework links barcode allocation, empirical performance, and antibody screening kit readouts, supporting five barcodes per target as a general starting point while allowing application-specific tuning to reduce over saturation risk.

Summary

AVITI24 with Teton workflow delivers reproducible, specific, and biologically accurate protein detection, validated through orthogonal and functional assays. Barcode allocation and number of cells sampled are key design variables directly influencing detection sensitivity and specificity, while plexity defines the panel design flexibility. Empirical benchmarking with TNF α -induced phospho-signaling revealed an inflection point near five barcodes per target, providing a useful reference for sensitivity and barcode allocation. Depending on experimental goals, tailor barcode allocation strategies to achieve strong performance—using more barcodes to improve confidence in low-abundance or highly regulated proteins.

Use the Custom Antibody Screening Kit readouts to confirm that increased allocation for selected targets does not push total signal density to a saturating level. The screening kit also serves as a complementary tool for testing antibody clones, vendors, and surface conditions to ensure optimal binding and reproducibility before a Teton CytoProfiling run.

Together, these results establish AVITI24 with Teton as a flexible, data-driven platform for protein detection. A practical framework for planning Teton protein detection is outlined that combines empirical validation, barcode allocation, and antibody screening to balance sensitivity, specificity, and plexity. This approach also maximizes the Teton protein assay success across diverse experimental systems.

To learn more, visit elementbiosciences.com

Reference

1. Lopez, T. et al. High-Throughput Multiomics Profiling of Model Systems Using the AVITI24 Platform. (May 2025): <https://doi.org/10.1101/2025.05.03.651997>.

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