

# Teton™ Custom Antibody Screening

## Guidance and Data Analysis

### Highlights

- Screen up to 96 custom primary antibodies
- Analyze primary antibody compatibility, cell morphology, and cellular localization in a single run
- Obtain results in 4 hours

### Introduction

The Teton Custom Antibody Screening Kit offers a rapid, scalable, and quantitative solution for validating the compatibility of your custom primary antibodies with a Teton Custom Add-On Protein Panel Assembly Kit using the AVITI24™ System.

The kit enables screening of up to two custom primary antibodies per well. A short, 4-hour antibody screening run delivers rapid assessment of up to 24 antibodies in a single 12-well Teton Slide Kit or 96 antibodies in a 48-well Teton Slide Kit.

Each antibody screening run provides a comprehensive cellular readout of target counts per cell for controls and custom primary antibodies along with cell morphology, cell confluency, and CellProfiler values. Comparison of the custom primary antibody counts relative to the negative control counts assist in confidently

selecting custom primary antibodies compatible with the Teton Custom Add-On Protein Panel.

The kit is compatible with a variety of cell lines such as adherent and suspension cell lines, immortalized cell lines, and primary cell lines.

The Teton Custom Antibody Screening Kit includes high-quality reagents sufficient for one antibody screening run. The kit includes a Teton Custom Screen Cartridge, a Teton Custom Antibody Screen Probe Kit with custom antibody probes, positive and negative controls, and a Teton Cell Paint Probe Kit with cell paint probes for cellular visualization.

### Workflow

Perform cell culture optimization on 96-well or 384-well glass plates and assess cell growth and health using bright field microscopy prior to culturing cells on a Teton slide kit. See the [Teton Optimization and Screening User Guide \(MA-00078\)](#) for protocol details and [Cell Culture Hub](#) for cell images.

Seed adherent or suspension cells directly onto 48-well or 12-well Teton slide kit at optimal seeding density and fix cells using validated protocols to ensure cell adhesion and confluency.

To prepare custom antibody probes, combine your primary antibodies with Teton Custom Antibody Screen Proteins, which are coordinating molecules for your custom primary antibodies.



**Figure 1.** Teton Custom Antibody Screening Kit workflow.

The probes are then combined with Teton Custom Antibody Screen Control to form ready-to-use Teton antibody pools integrated with positive and negative controls.

The off-instrument workflow takes ~2 hours and consists of preparing your antibody probes, incubating your samples with the Teton antibody pools and Teton cell paint probes, and assembling the Teton flow cell. Load the Teton Custom Screen Cartridge and the assembled flow cell onto an AVITI24 System to initiate the antibody screening run, and analyze results in 4 hours (Figure 1).

## Primary Antibody Requirements

The Teton Custom Antibody Screening Kit is compatible with rabbit-derived monoclonal or polyclonal primary antibodies. Using non-rabbit host custom primary antibodies can cause false positive results. For optimal performance, monoclonal antibodies are preferred.

To ensure consistency and best results, select antibodies that are validated by Western blot and an additional method such as immunocytochemistry/immunofluorescence or flow cytometry. Antibodies validated using multiple methods tend to perform more reliably with the Teton assay.

Element recommends testing multiple custom primary antibodies to the same target to ensure successful identification of usable custom primary antibodies for the Teton run. Ideally, test custom

primary antibodies in replicates from various vendors and different lot numbers to increase hit probability per screen.

## Experimental Setup

As a best practice, always perform the screening 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 flow cell.

For optimal performance with the Teton Custom Antibody Screening Kit, first optimize the seeding density for your cell line by performing cell culture optimization using 96-well or 384-well glass plates and bright field microscopy. Use the optimal glass plate seeding density to culture cells on a Teton slide kit to target 30–70% confluency based on your cell type for an antibody screening run.

Table 1 shows an example experimental setup for custom antibody screening using different primary antibodies with multiple cell lines. You can use the Teton Custom Antibody Screening Kit on multiple cell lines for targets that are cell line specific or regulated to confirm probe specificity or probe efficacy across multiple cell lines of interest.

Figure 2 shows the results of an antibody screening run across multiple cell lines.

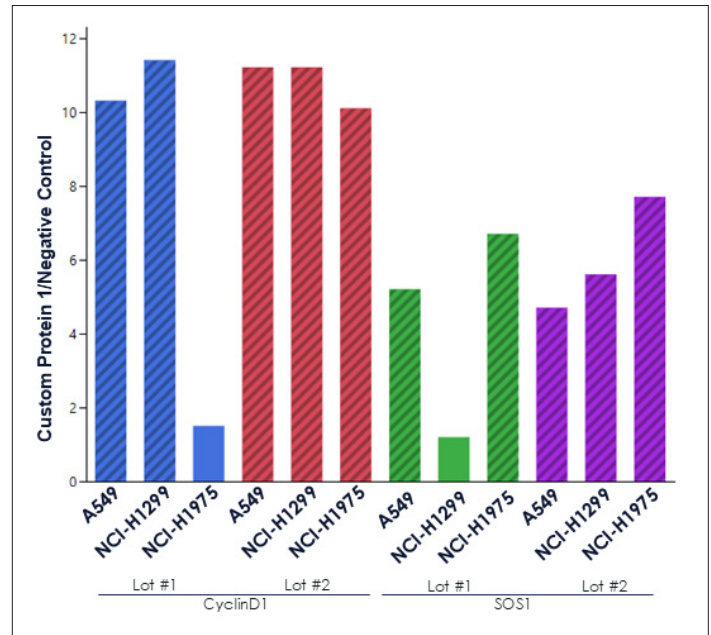
Well	1	2
A	<b>Cell Line: A549</b> Custom Antibody 1: CyclinD1 Lot 1 Custom Antibody 2: phospho-CyclinD1 Lot 1	<b>Cell Line: A549</b> Custom Antibody 1: CyclinD1 Lot 2 Custom Antibody 2: phospho-CyclinD1 Lot 2
B	<b>Cell Line: A549</b> Custom Antibody 1: SOS1 Lot 1 Custom Antibody 2: Ras Lot 1	<b>Cell Line: A549</b> Custom Antibody 1: SOS1 Lot 2 Custom Antibody 2: Ras Lot 2
C	<b>Cell Line: NCI-H1975</b> Custom Antibody 1: CyclinD1 Lot 1 Custom Antibody 2: phospho-CyclinD1 Lot 1	<b>Cell Line: NCI-H1975</b> Custom Antibody 1: CyclinD1 Lot 2 Custom Antibody 2: phospho-CyclinD1 Lot 2
D	<b>Cell Line: NCI-H1975</b> Custom Antibody 1: SOS1 Lot 1 Custom Antibody 2: Ras Lot 1	<b>Cell Line: NCI-H1975</b> Custom Antibody 1: SOS1 Lot 2 Custom Antibody 2: Ras Lot 2
E	<b>Cell Line: NCI-H1299</b> Custom Antibody 1: CyclinD1 Lot 1 Custom Antibody 2: phospho-CyclinD1 Lot 1	<b>Cell Line: NCI-H1299</b> Custom Antibody 1: CyclinD1 Lot 2 Custom Antibody 2: phospho-CyclinD1 Lot 2
F	<b>Cell Line: NCI-H1299</b> Custom Antibody 1: SOS1 Lot 1 Custom Antibody 2: Ras Lot 1	<b>Cell Line: NCI-H1299</b> Custom Antibody 1: SOS1 Lot 2 Custom Antibody 2: Ras Lot 2

**Table 1.** Example of an experimental setup for a 12-well Teton Slide Kit with multiple cell lines for ERK pathway antibodies. Column 2 is a duplicate of Column 1 to allow for testing in replicates.

## Specific Applications

In addition to screening for custom primary antibody compatibility, the Teton Custom Antibody Screening Kit also enables testing cellular regulation of target proteins and comparisons across specific cell lines using custom primary antibodies. This testing aids in determining if the antibodies are responsive to treatments known to regulate the impacted proteins.

An example of regulation is adding lambda phosphatase (LPP) to permeabilized cells and incubating for 1 hour at 30°C to reduce phosphoprotein levels. You can easily design an experiment to confirm specificity of antibodies targeting phosphoproteins as outlined in Table 2 and quickly select custom primary antibodies that are selectively detecting the phosphoprotein of interest (Figure 3).



**Figure 2.** Example of custom primary antibodies screened across multiple cell lines as described in Table 1. Custom primary antibodies with higher normalized counts per cell (indicated in diagonal stripes) are considered PASS for use with Teton Custom Add-On Protein Panel. Antibodies with lower counts (indicated as solid bar) are considered poor performers and not recommended for use with the Teton Custom Add-On Protein Panel.

Well	1	2
A	<b>Wild Type Cells</b> Custom Antibody 1: phospho-PKA Lot 1 Custom Antibody 2: phospho-HSP27 Lot 1	<b>LPP Treated Cells</b> Custom Antibody 1: phospho-PKA Lot 1 Custom Antibody 2: phospho-HSP27 Lot 1
B	<b>LPP Treated Cells</b> Custom Antibody 1: phospho-PKA Lot 2 Custom Antibody 2: phospho-HSP27 Lot 2	<b>Wild Type Cells</b> Custom Antibody 1: phospho-PKA Lot 2 Custom Antibody 2: phospho-HSP27 Lot 2
C	<b>Wild Type Cells</b> Custom Antibody 1: phospho-PKA Lot 3 Custom Antibody 2: phospho-HSP27 Lot 3	<b>LPP Treated Cells</b> Custom Antibody 1: phospho-PKA Lot 3 Custom Antibody 2: phospho-HSP27 Lot 3
D	<b>LPP Treated Cells</b> Custom Antibody 1: phospho-EGFR Lot 1 Custom Antibody 2: phospho-cFOS Lot 1	<b>Wild Type Cells</b> Custom Antibody 1: phospho-EGFR Lot 1 Custom Antibody 2: phospho-cFOS Lot 1
E	<b>Wild Type Cells</b> Custom Antibody 1: phospho-EGFR Lot 2 Custom Antibody 2: phospho-cFOS Lot 2	<b>LPP Treated Cells</b> Custom Antibody 1: phospho-EGFR Lot 2 Custom Antibody 2: phospho-cFOS Lot 2
F	<b>LPP Treated Cells</b> Custom Antibody 1: phospho-EGFR Lot 3 Custom Antibody 2: phospho-cFOS Lot 3	<b>Wild Type Cells</b> Custom Antibody 1: phospho-EGFR Lot 3 Custom Antibody 2: phospho-cFOS Lot 3

**Table 2.** Example of an experimental setup for a 12-well Teton Slide Kit with cells treated with LPP to screen for antibodies targeting phosphoprotein targets.

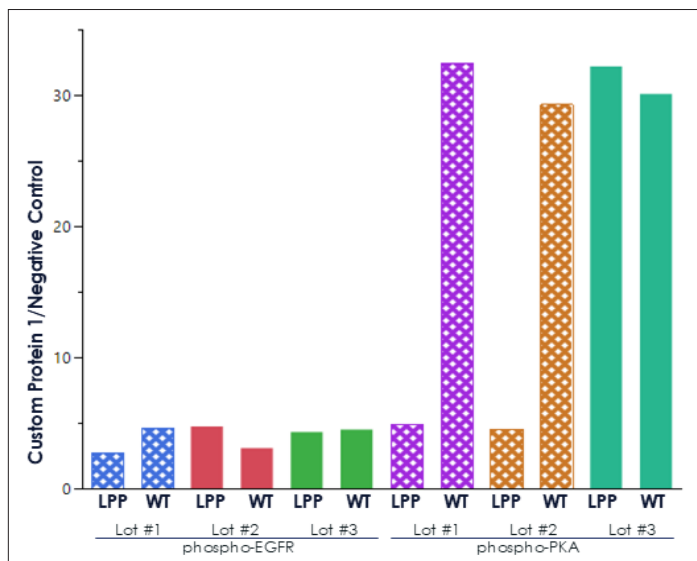
## Data Analysis

The AVITI Operating Software simplifies data analysis and provides real-time, onboard cell segmentation, cell assignment, and morphology reporting in 4 hours for an antibody screening run. Element CytoCanvas™ is an easy-to-use, free visualization software that provides off-instrument interactive data visualization of your cytoprofilng data.

To identify compatible custom primary antibodies, review the cytoprofilng run output files (AntibodyScreenKitReport.csv and RawCellStats.csv):

- Analyze the target counts/negative control counts per cell (normalized values) in the AntibodyScreenKitReport.csv file.
  - Custom primary antibodies that exhibit normalized values of > 4 for adherent cells and > 8 for suspension cells are considered a PASS.
  - If normalized values are 1-4 for adherent cells or 1-8 for suspension cells, the custom primary antibody requires further inspection to determine antibody compatibility. Repeat the antibody screening run and testing for regulation, if applicable, to confirm values are within this range of signal.
- Proceed with caution, if similar results are observed indicating that the protein target may be expressed at a low level. Test an alternative custom primary antibody or confirm biological relevance or response using regulation or knockout cell lines.
- If normalized values are ≤ 1, the custom primary antibody is considered a FAIL.
- Confirm that negative control counts are not higher than positive control counts, indicating that kit controls are functioning as expected.
- Review the Cell Count, Cell Diameter, and Cell Area listed in the AntibodyScreenKitReport.csv file.

Additionally, assess custom primary antibody localization using CytoCanvas to confirm that localization is as expected. Review the RawCellStats.csv file for CellProfiler metrics such as cell morphology and cell diameter.



**Figure 3.** Example data of LPP treated flow cell as described in Table 2. Crosshatched bars represent custom primary antibodies that are considered passing in the antibody screening run demonstrating regulation with LPP.

## Summary

Whether you are screening across multiple primary antibodies or exploring antibody regulation, the entire Teton Custom Antibody Screening Kit workflow lets you screen up to 96 custom primary antibodies in less than 7 hours. Easily couple the primary antibody of interest to high-quality Teton custom screen protein probes and apply the probes along with Teton cell paint reagents to cultured cells followed by flow cell assembly and imaging on an AVITI24 System.

With automated onboard primary data analysis and off-instrument interactive data visualization with CytoCanvas, confidently select compatible primary antibodies for use with the Teton Custom Add-On Protein Panel within a day.

To learn more, visit [elementbiosciences.com](https://elementbiosciences.com)

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