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Technical Note

The Simple Western Assay for Residual Benzonase Quantification

Automated and Sensitive Benzonase Quantification in Whole-Cell Lysates

In downstream bioprocessing workflows like the manufacture of viral vaccines for infectious diseases and viral vectors for cell and gene therapy, impurities resulting from host cell DNA are removed with Benzonase® treatment, an endonuclease encoded by NucA. Like other process additives, the removal of Benzonase is an essential purification step that is mandated by regulatory agencies as residual Benzonase may impact patient safety.[1]

Simple Western™ from ProteinSimple is the only fully automated capillary immunoassay platform that reproducibly quantifies trace amounts of Benzonase in the low picogram range. While the ELISA workflow for Benzonase detection involves many manual washing and incubation steps,[2] Simple Western offers reproducible and sensitive Benzonase quantification following simple sample preparation and plate loading (FIGURE 1).

In addition to a streamlined workflow, Simple Western provides significant advantages over ELISA, making Simple Western a scalable solution in biomanufacturing workflows and improving in-process development (TABLE 1). For example, Simple Western needs only 3 µL of sample, compared to the 100 µL or more needed for ELISA,[2] minimizing impact on final product titers. Simple Western even provides reproducible quantification in complex sample types like whole-cell lysates.

Unlike traditional ELISA workflows, Simple Western provides separation of target proteins by molecular weight (size) or pI (charge), allowing for the identification of antibody cross-reactivity that would otherwise go unnoticed with ELISA. Furthermore, Simple Western produces no liquid waste, whereas ELISA generates more than 1 L of liquid waste per run, including the use of toxic chemicals like sulfuric acid.[2]

In this Technical Note, we describe a Simple Western assay to quantify trace amounts of Benzonase in HEK293T whole-cell lysate. With reproducible quantification of Benzonase at concentration below 1 ng/mL, Simple Western overcomes the challenges of traditional ELISA without compromising sensitivity and reduces hands-on time to just 30 minutes for sample preparation and plate loading making it possible to integrate at-line or near-line and achieve fast, actionable results.

FIGURE 1. Comparison of ELISA and Simple Western Workflows for Monitoring Residual Benzonase

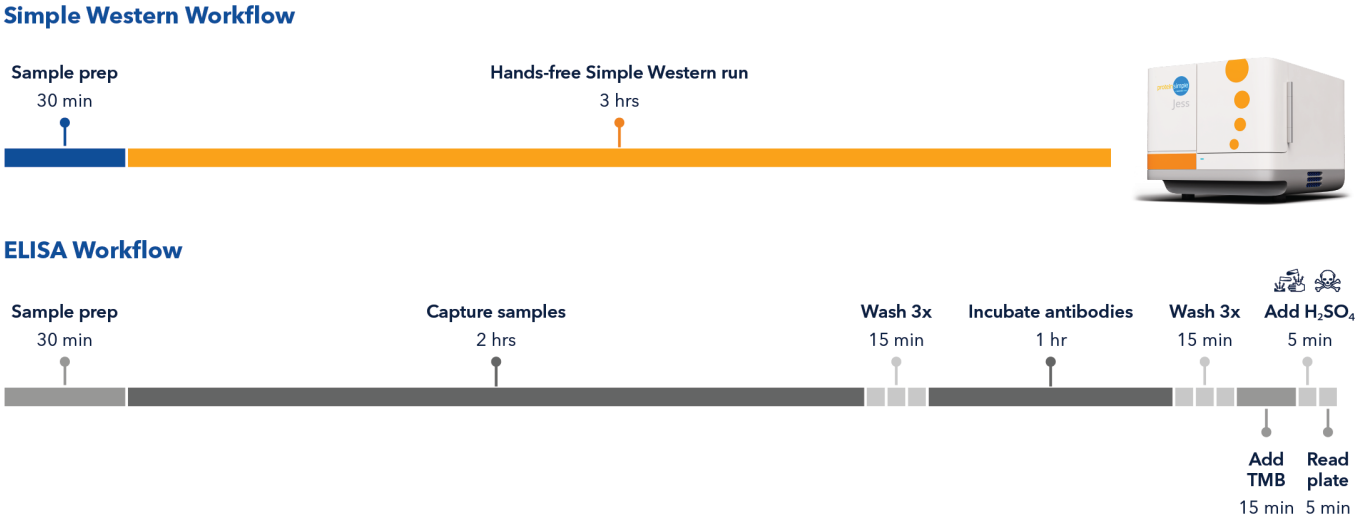

TABLE 1. Comparison of ELISA[2] and Simple Western Assays for Monitoring Residual Benzonase

	ELISA	Simple Western
Sample requirement	100 µL	3 µL
Hands-on time	1.5 hours (with intermissions)	30 minutes (sample preparation & loading)
Run time	5 hours	3 hours
Liquid waste	1 L wash buffer + 150 mL H_2SO_4	None
Size separation	No	Yes

Materials and Methods

Materials and reagents used in this study are listed in TABLE 2. The primary anti-Benzonase antibody was prepared in Antibody Diluent 2 at a 1:25 dilution (0.02 mg/mL final concentration), and the secondary antibody was used at the ready-to-use (RTU) stock concentration.

To establish a standard curve for residual Benzonase quantification, a 6-point 5-fold titration of recombinant Benzonase was prepared starting at 2500 ng/mL with a four-parameter logistic (4PL) regression analysis.

To establish high and low quality control (QC) samples, recombinant Benzonase was prepared at 400 ng/mL and 10 ng/mL final concentrations, respectively. To quantify trace amounts of Benzonase, recombinant Benzonase was prepared at final concentrations (ng/mL) of 1, 0.9, and 0.8. All samples were prepared in a HEK293T whole-cell lysate background with a constant concentration of 10 µg/mL.

All samples were denatured for 5 minutes at 95 °C and were analyzed on Jess™, a Simple Western instrument, using the 12-230 kDa Separation Module with default conditions and settings.

TABLE 2. Materials and Reagents Used in This Study

Item	Vendor	Part Number
Jess	Bio-Techne	004-650
12-230 kDa Separation Module		SM-W001
EZ Standard Pack 1		PS-ST01EZ-8
Anti-Rabbit Detection Module		DM-001
Recombinant S. marcescens Benzonase protein		10038-NA
Rabbit Benzonase® Nuclease Monoclonal Antibody		MAB100632
HEK293T whole-cell lysate	Origene	LY500001

Testing the Simple Western Benzonase Assay in HEK293T Whole-Cell Lysates

To establish the Benzonase assay on Simple Western, recombinant Benzonase was prepared in a serial dilution series as described in the Materials and Methods, and this dilution series was analyzed on Jess using the anti-Benzonase antibody. The results from this analysis showed a strong signal peak corresponding to Benzonase with an apparent molecular weight of 36 kDa and the signal diminshed with decreasing Benzonase concentration in each sample (FIGURE 2).

Despite the presence of HEK293T whole-cell lysate at a constant concentration of 10 µg/mL in all samples, no other peaks were observed on Simple Western using the anti-Benzonase antibody (FIGURE 2). Thus, the Simple Western assay for Benzonase detection is highly specific, even in complex sample types like HEK293T whole-cell lysate used here.

To generate a standard curve for Benzonase quantification, the average peak area was calculated from the Benzonase serial dilution series and plotted against the corresponding sample concentration. Then, a 4PL regression analysis was applied to create a line of fit (FIGURE 2, inset). From this standard curve, we interpolated X values from the mean peak areas, which resulted in concentrations recovered within 10% of the expected value (TABLE 3).

Next, we analyzed high QC and low QC control samples containing Benzonase concentrations of 400 ng/mL and 10 ng/mL, respectively (FIGURE 3). With the resulting average peak areas (FIGURE 3, inset), we interpolated Benzonase concentrations and recovery. All samples recovered within 11% of expected values (TABLE 4).

FIGURE 2. Standard Curve for Benzonase Quantification on Simple Western

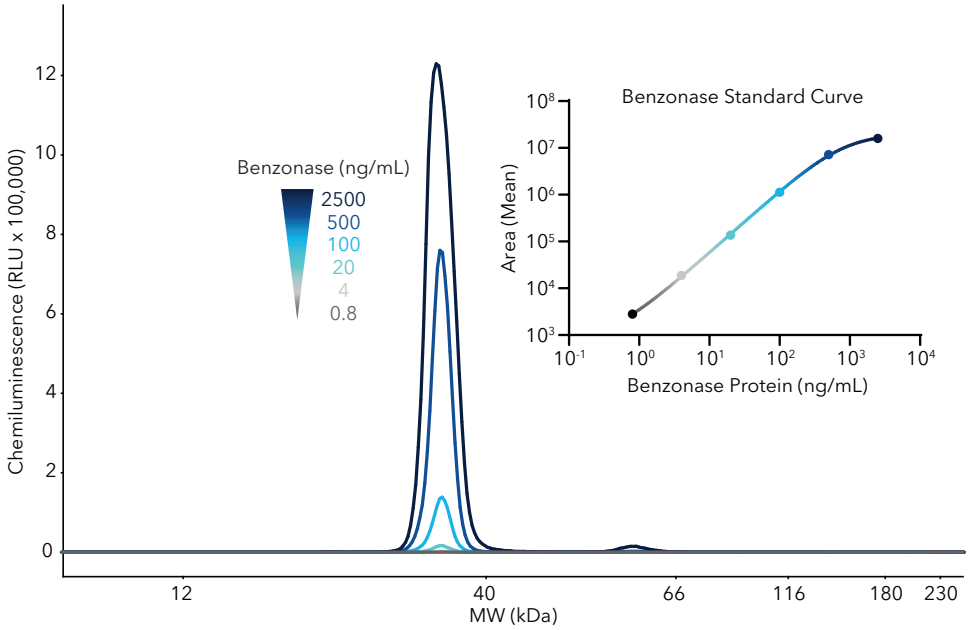

FIGURE 3. Simple Western Analysis of High and Low QC Samples

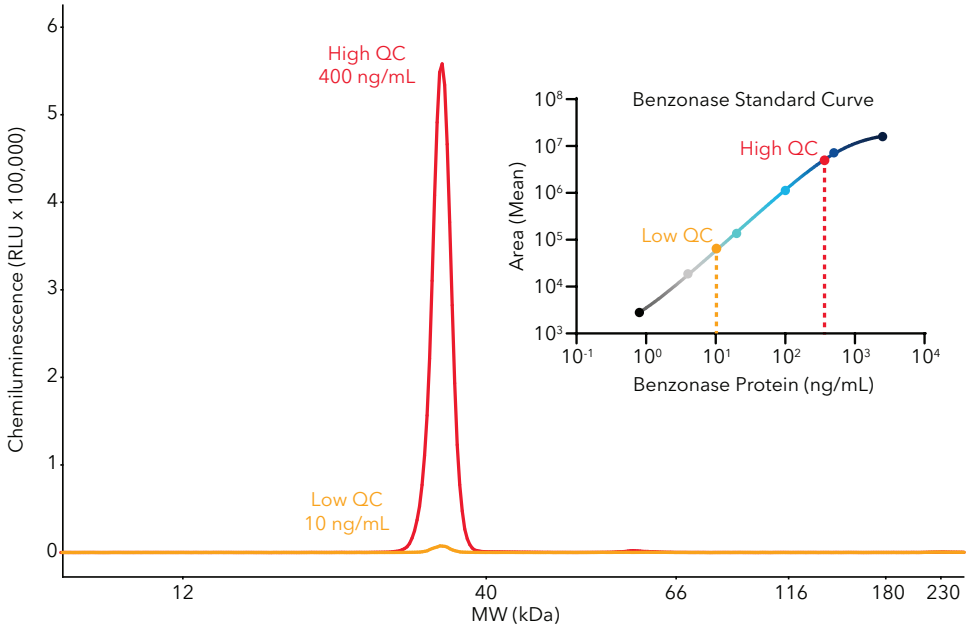

TABLE 3. Benzonase Standard Curve

Benzonase (ng/mL)	Recovery (%)
2500	91.9
500	106.4
100	98.1
20	95.9
4	103.9
0.8	99.3

Benzonase recovery was interpolated using the standard curve resulting from Simple Western analysis of a serial dilution series of recombinant Benzonase prepared in HEK293T whole-cell lysate.

TABLE 4. Quantification of High and Low QC Samples

Benzonase (ng/mL)	Interpolation (ng/mL)	Recovery (%)
400	365.7	91.4
10	11.1	110.8

Benzonase concentrations and recovery were interpolated using the average peak areas resulting from Simple Western analysis of HEK293T whole-cell lysate spiked with high and low QC samples, corresponding to 400 ng/mL and 10 ng/mL of recombinant Benzonase, respectively.

To test the lower limit of quantification, recombinant Benzonase was spiked at concentrations of 1, 0.9, and 0.8 ng/mL in a HEK293T whole-cell lysate background and these samples were analyzed on Jess. Using the standard curve, we interpolated Benzonase concentration from the average peak areas. All samples showed Benzonase recovery within 20% of the expected values (TABLE 5). These results demonstrate that Simple Western can reproducibly quantify Benzonase at concentrations as low as 1 ng/mL with a CV of 3.4%, even in complex sample types, like HEK293T whole-cell lysate used here. Because Simple Western requires only 3 µL of sample, 1 ng/mL corresponds to just 3 pg of Benzonase.

TABLE 5. Quantification of Residual Benzonase

Benzonase (ng/mL)	Interpolation (ng/mL)	Recovery (%)
1	1.05	104.5
0.9	0.75	83.3
0.8	0.66	82.4

Benzonase concentrations and recovery were interpolated using the average peak areas (*n*=2) resulting from Simple Western analysis of HEK293T whole-cell lysate spiked with Benzonase at concentrations (ng/mL) of 1, 0.9, and 0.8.

Ditch ELISA for Automated and Reproducible Benzonase Quantification for Downstream Bioprocessing Workflows

Here, we demonstrate that Simple Western reproducibly and quantitatively characterizes Benzonase at concentrations of at least 1 ng/mL in complex sample types like HEK293T whole-cell lysate, meeting regulatory standards for development of viral vaccines for infectious disease and viral vectors for cell and gene therapy applications.[1] Unlike the traditional ELISA assay,[2] this Simple Western assay for residual Benzonase quantification is fully automated following simple sample preparation and plate loading. While samples were prepared manually prior to Simple Western analysis, automated liquid handlers like the CyBio Felix from Analytik Jena may be implemented to automate sample preparation and plate loading to streamline workflows even further.[3] Taken together, we anticipate that the Simple Western assay for monitoring residual Benzonase will optimize downstream bioprocessing workflows that rely on Benzonase treatment, including the biomanufacturing of vaccines for combating infectious disease and viral vectors for cell and gene therapy.

References

1. Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infections Disease Indications, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research, 2010
2. Benzonase® ELISA Kit II, User Guide, EMD Millipore
3. Automated Solution for Simple Western™ Jess - A High-Throughput Western Blotting Technique, Application Note, Analytik Jena

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