Application Note

Rapid Endonuclease Quantification

Automated Process Residual Characterization with Ella™

Endonuclease is a non-specific nuclease that degrades DNA and RNA by breaking phosphodiester bonds without any proteolytic activity. This makes endonuclease the ideal enzyme for various applications where nucleic acid digestion of the host cell is necessary. These applications include viral vector preparations, vaccine products, and other biotherapeutics[1]. Once the host-cell nucleic acid is digested, it is important to verify that residual endonuclease has been removed from the final product. To that end, the Simple Plex™ Endonuclease assay was developed as a selective and sensitive method for detecting residual endonuclease impurities in bioprocess samples.

High Sensitivity, Broad Dynamic Range, and Accuracy with Simple Plex Endonuclease Assay

The Simple Plex Endonuclease assay has a 4-log dynamic range with high sensitivity to reliably detect residual endonuclease impurities in process samples (FIGURE 1).

Recovery testing (FIGURE 2) and dilution linearity (FIGURE 3) were evaluated for accuracy and specificity using bioprocess samples.

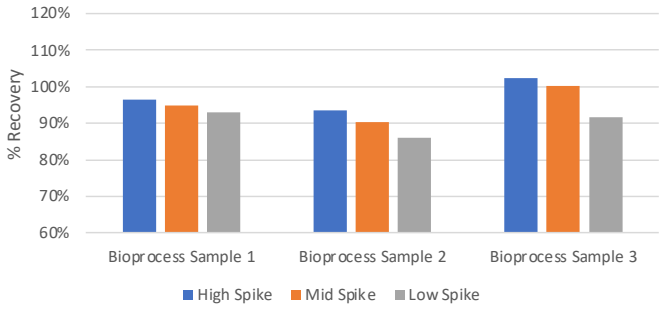

FIGURE 2. Endonuclease was spiked into 3 different matrices representing different steps of the bioprocess workflow. Recovery percentages at three spiked concentrations (high, mid, low) yielded good recovery, meeting specifications.

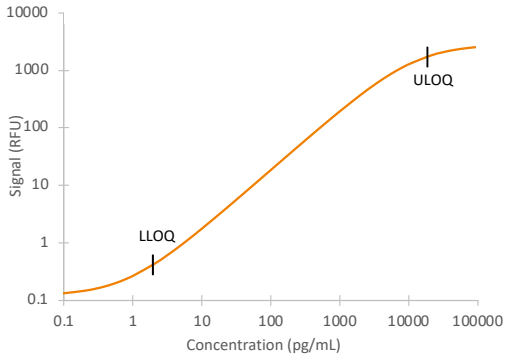

FIGURE 1. Standard curve range with a lower limit of quantification (LLOQ) of 1.97 and an upper limit of quantification (ULOQ) of 18,750 pg/mL.

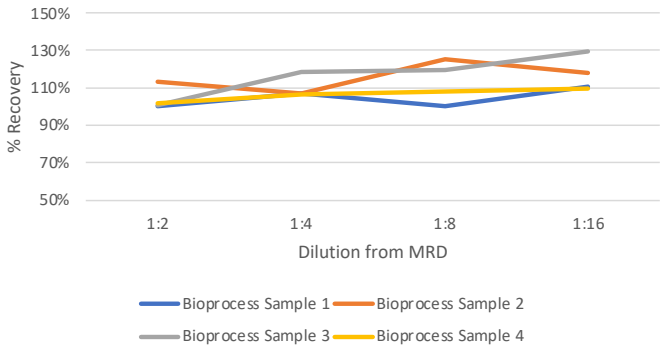

FIGURE 3. Four bioprocess samples containing endonuclease were diluted within the range of the Simple Plex assay. Mean recovery for all samples was between 101% and 116%, which meet specifications.

Automated Workflow and Microfluidic Design Yield High Inter-Assay Precision and Help Save Time

The automated, microfluidic design of the Simple Plex assay cartridge eliminates user-introduced variability providing high precision and reproducibility. To assess inter-assay precision, we evaluated endonuclease presence in a bioprocess sample that was loaded repeatedly across multiple cartridges and cartridge lots, by four operators. Even at low picogram levels, the results were reproducible regardless of operators, cartridge, or cartridge lot, with an overall coefficient of variance (CV) of 10.5% (FIGURE 4).

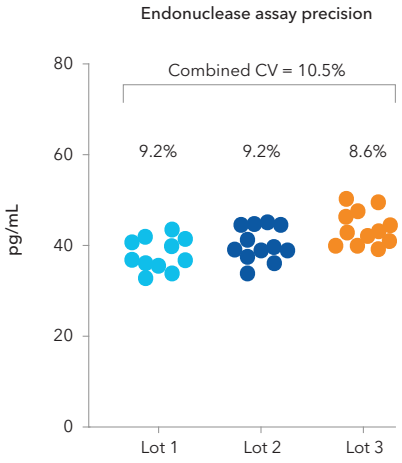

FIGURE 4. Multiple preparations of a bioprocess sample were loaded by four operators across multiple cartridges from three independent cartridge lots.

The unique design of the Simple Plex platform not only simplifies the workflow and speeds up the time from sample preparation to result, but also has built-in factory standard curves. Once samples have been diluted and loaded into the Ella cartridge, the hands-free automated process takes 90 minutes for Ella to provide fully analyzed, triplicate results per sample.

The Simple Plex Endonuclease Assay Detects both Benzonase® and Denarase®

To confirm the applicability of the Simple Plex Endonuclease assay to commercially available products, we evaluated detectability and dilutional linearity Benzonase® and Denarase®. The results demonstrate good performance for both products across the dynamic range of the assay (FIGURE 5).

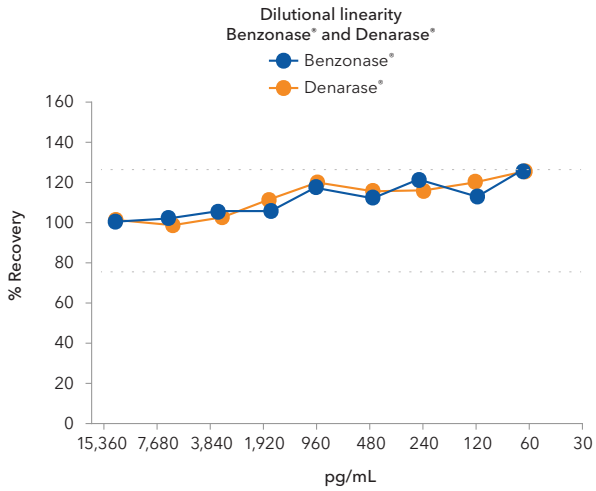

FIGURE 5. Serial dilution of both Benzonase® and Denarase® across the dynamic range of the assay.

Conclusion

The Simple Plex Endonuclease assay on Ella provides an automated highly sensitive solution for detecting residual impurities in bioprocess samples.

The Simple Plex Endonuclease assay is available in 16x1 and 32x1 cartridge formats. Additional Simple Plex assays catered to simplify and support the bioprocess workflow include HEK-HCP and CHO-HCP assays, AAV titer assays, and HIV-1p24[2].

References

1. Benedik, MJ and Strych, U. (1998) FEMS Microbiol Lett. 165:1.
2. Van Manen-Brush et al. (2020) Biotechniques, 69(3):186-192.