J.T.Baker® Endonuclease Detection using Enzyme linked immunosorbent Assay (ELISA)

BACKGROUND

J.T.Baker® Endonuclease is a bioengineered enzyme, based on native endonuclease *Serratia marcescens*, that effectively degrades both single stranded and double stranded DNA and RNA in harvest material from cell lysate or supernatant produced during biologics manufacturing processes such as AAV or LV. The enzyme is a sugar nonspecific hydrolase that degrades phosphodiester bonds, resulting in smaller and more soluble fragments[1]. This in turn can reduce aggregation and precipitation from undigested nucleic acids for easier downstream processing. A critical aspect of this process is the detection of residual endonuclease in the downstream product. If present in the final product, it can potentially degrade therapeutic genetic material, leading to reduced potency or unwanted immune responses in patients. Therefore, reliable detection and quantification of residual endonuclease is essential for confirming the quality and safety of final drug product. This technical note provides evidence that a commercially available Enzyme-linked Immunosorbent Assays (ELISA's) may be used for detection of J.T.Baker® Endonuclease to ensure residual levels are within acceptable limits.

OVERVIEW OF METHOD

For this study, samples of J.T.Baker® Endonuclease were evaluated using CYGNUS Technologies EndonucleaseGTP® F960 ELISA Kit, VWR part number MSPP-F960, to demonstrate the ability of a commercially available assay to detect and accurately quantify residual levels of endonuclease impurities in downstream process samples.

The general procedure for this ELISA consists of incubating samples containing residual amounts of J.T.Baker® Endonuclease with horseradish peroxidase (HRP) labeled anti-endonuclease polyclonal antibody in 12 x 8 well microtiter strips coated with unconjugated anti-endonuclease polyclonal antibody. After incubation, the wells are washed using a buffered saline solution. Following the final wash, a chromogenic substrate, 3,3',5,5' Tetramethylbenzidine (TMB), is added to the wells. TMB chemically reacts with the HRP enzyme to generate a detectable signal that is measured spectrophotometrically[2]. The intensity of the color produced correlates to the concentration of endonuclease in the sample, providing quantitative results.

ASSAY PROTOCOL

1. A stock solution of endonuclease (0.188 mg/mL) was serially diluted to prepare stock dilutions for the test. A 10X dilution was first prepared by diluting 15 µL of endonuclease at a concentration of 0.188 mg/mL in 135 µL Phosphate buffered saline (PBS) and mixed by vortex for 15 seconds. The initial 10X stock solution was serially diluted in the same manner to prepare subsequent stock solutions at 1:100, 1:1,000, 1:10,000, and 1:100,000.
2. Samples were prepared by diluting the stock solutions described in Table 1.

Target Concentration	Serial Dilution Factor	Endonuclease Dilution Volume (µL)	PBS Volume (µL)
0 ng/mL	N/A	0	100
0.31 ng/mL	1:100,000	16.5	83.5
0.63 ng/mL	1:100,000	33.5	66.5
1.25 ng/mL	1:100,000	66.5	33.5
2.5 ng/mL	1:10,000	13.3	86.7

5 ng/mL	1:10,000	26.6	73.4
10 ng/mL	1:10,000	53.19	46.81
20 ng/mL	1:1,000	10.64	89.4

Table 1: Endonuclease sample preparation dilutions

3. Reagents were equilibrated to room temperature and prepared according to Cygnus EndonucleaseGTP® Product Insert CYG-LBL-00387.
4. Spectrophotometer was configured to read dual wavelength at 450 nm for the test wavelength and ~650 nm for the reference.
5. All standards, controls and samples were prepared in duplicate at concentrations spanning the range of the assay at 0 ng/mL, 0.31 ng/mL, 0.63 ng/mL, 1.25 ng/mL, 2.5 ng/mL, 5 ng/mL, 10 ng/mL, and 20 ng/mL.
6. 100 µL of anti-EndonucleaseGTP®:HRP was added into each well.
7. 50 µL of standards, controls, and samples were pipetted into each designated well. Wells were covered and incubated on shaker (400 – 600 rpm) for 60 minutes at room temperature, 24°C ± 4°C.
8. Microtiter wells were emptied and then washed 4X using Tris buffered saline solution, prepared per manufacturer's instructions. Note: Use plate washer or fill wells using a laboratory wash bottle. If washing manually, blot wells gently and firmly over particulate free, absorbent paper to remove residual liquid.
9. 100 µL TMB substrate was added to wells. The plate was incubated at room temperature for 30 minutes without shaking.
10. After 30-minute incubation, the reaction was stopped by pipetting 100 µL Stop Solution into the wells.
11. The absorbance of each well was measured at 450/650 nm within 30 minutes after the reaction was ended.

RESULTS

Samples were analyzed per Cygnus EndonucleaseGTP® ELISA kit instructions, CYG-LBL-00387, in duplicate and concentrations determined using sample standard curve fit. Precision on duplicate samples were within the expected variation of < 10% to meet the acceptance criteria of the assay. Results for mean delta optical density (OD) and standard deviation between duplicates (STDEV) for standards and samples with known residual levels of J.T.Baker® Endonuclease are found in Table 2. Endonuclease concentrations were calculated from the standard curve depicted in Figure 1.

Concentration	Cygnus kit standards Mean Delta OD (nm)	Standards STDEV	J.T.Baker® Endonuclease Mean Delta OD (nm)	Samples STDEV
0 ng/mL	0.078	0.002	0.078	0.006
0.31 ng/mL	0.108	0.002	0.1	0.002
0.63 ng/mL	0.139	0.011	0.118	0.007
1.25 ng/mL	0.206	0.016	0.172	0.009
2.5 ng/mL	0.316	0.004	0.283	0.009
5 ng/mL	0.532	0.036	0.512	0.015
10 ng/mL	0.911	0.055	0.847	0.007
20 ng/mL	1.609	0.075	1.61	0.059

Table 2: Absorbance measurement and precision results for J.T.Baker® Endonuclease

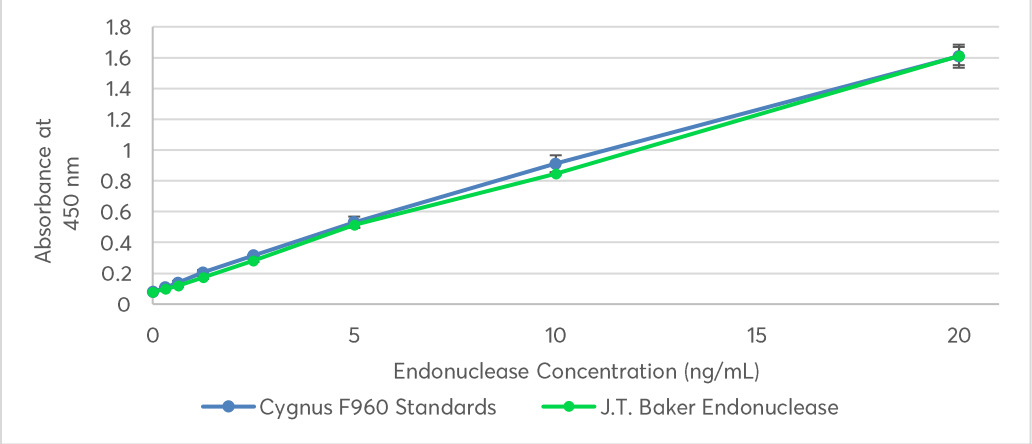

Figure 1: Detection of residual levels of J.T.Baker® Endonuclease by absorbance at 450 nm versus Cygnus F960 standards.

CONCLUSION

The results obtained from the assay outlined in this technical note are acceptable with the established methodological framework and confirms the reliability of commercially available ELISA kits to quantitatively determine residual levels of J.T.Baker® Endonuclease. Use of commercially available ELISA kits to detect residual levels of J.T.Baker® Endonuclease supports the optimization of purification processes, aids in process control, and is useful in routine quality assurance and product release testing.

1. Nestle, M. and Roberts, W.K. (1969) An extracellular nuclease from Serratia marcescens. J. Biol. Chem. 244, 5213 – 5218
2. **Source**: *Cygnus EndonucleaseGTP® Product Insert CYG-LBL-00387 Rev:05 Eff: 24 Apr 2023*