A custom method for endotoxin quantification

NanoDrop One/One^{C} Spectrophotometer

Introduction

The Thermo Scientific™ Pierce™ Chromogenic Endotoxin Quant Kit is an endpoint chromogenic detection assay based on the *Limulus* amebocyte lysate (LAL) assay. When *Limulus* amebocytes encounter lipopolysaccharides (LPS) in gram-negative bacteria, an enzymatic clotting cascade is activated to neutralize the pathogen.[1] Applying this cascade to the chromogenic assay, the activated clotting enzyme catalyzes the release of p-nitroaniline (pNA) from the synthetic substrate, Ac-Ile-Glu-Ala-Arg-pNA.[2] The released pNA produces a yellow color that can be quantified with the Thermo Scientific NanoDrop™ One/One^{C} Microvolume UV-Vis Spectrophotometer at 405 nm (Figure 1). By creating a standard curve that relates absorbance intensity to the endotoxin concentration, the amount of endotoxin present in an unknown sample can be quantified. As gram-negative bacteria are used in recombinant DNA technologies, endotoxin contamination is common.[3] The presence of endotoxin has been shown to affect inflammation and toxicity of biomaterials and reduce transfection efficiencies, thus, accurate quantitation is crucial.[4–7]

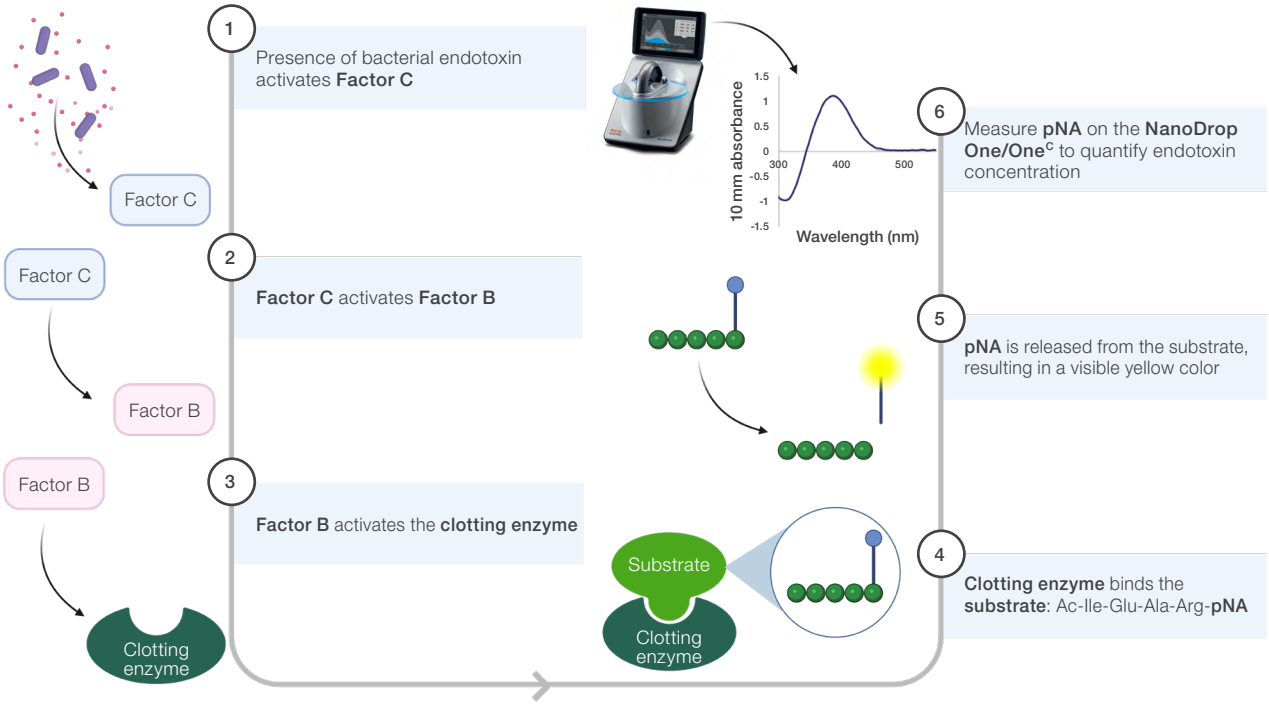

Figure 1: Activated clotting cascade catalyzes the release of p-nitroaniline (pNA) for spectrophotometric quantification at 405 nm. Created with BioRender.com.

Experimental procedures

NanoDrop One custom method

A custom method using a standard curve was created on the NanoDrop One/One^C PC Control Software to quantify endotoxin concentration in the Visible range (350–850 nm). The Custom Method file can be downloaded from the software section of the NanoDrop homepage at thermofisher.com/nanodrop. The method includes an analysis wavelength at 405 nm and a baseline correction at 750 nm. With an analysis wavelength selected, the NanoDrop One/One^C Software generates a standard curve based on the absorbance of the standards at that wavelength. The method also included the selection of Automated Pathlength to ensure the best pathlength was used depending on the absorbance intensity at the analysis wavelength to prevent detector saturation. Standards and samples were measured in 2 µL volumes using the microvolume pedestal on the NanoDrop One instrument.

Pierce Chromogenic Endotoxin Quant Kit

The Pierce Chromogenic Endotoxin Quant Kit (Thermo Scientific, A39553) includes the necessary contents to quantify endotoxin. The kit includes *E. coli* strain 0111:B4 endotoxin standard, amebocyte lysate from *Limulus*, and a chromogenic, synthetic substrate. In lieu of endotoxin-containing samples, the *E. coli* standard was diluted to 0.45 EU/mL (endotoxin unit per milliliter) and 0.15 EU/mL. The experimental procedure was followed as stated in the Endotoxin Quant Kit User Guide, with some minor deviations. The standard curve was created to include four high-range serial dilutions, 1.0 EU/mL, 0.5 EU/mL, 0.25 EU/mL, and 0.125 EU/mL. Since the NanoDrop One microvolume pedestal was used for the experiment, 1.5 mL microcentrifuge tubes were used in place of a 96-well microplate. A blank solution was prepared in conjunction with the samples and standards but without the addition of endotoxin; the blank contained endotoxin-free water, amebocyte lysate, chromogenic substrate, and stop solution (25% acetic acid). Preparing the blank solution in this manner ensures accurate pNA absorbance at 405 nm.

Results

The endotoxin standard curve displayed excellent linearity with R^2 = 0.9995, as shown in Figure 2. The amount of absorbance measured after the reaction was stopped is directly correlated with the endotoxin concentration.

Once the standard curve was measured, the NanoDrop One/One^C Software calculated the unknown sample concentration based on the absorbance relative to the standard curve (Table 1). The standard deviation was less than or equal to 0.011A and the coefficient of variation (%CV) ranged from 1.03% to 2.80%, based on triplicate measurements. Since the NanoDrop One/One^C Software automatically applies a blank absorbance correction, tedious calculations outside of the software are not required.

Conclusions

Endotoxin contamination is a common problem in biotechnical and pharmaceutical applications, causing cellular toxicity, a pyrogenic immune response, and reduced transfection efficiencies. The reliability of the NanoDrop One/One^C instrument for accurate and reproducible endotoxin quantitation has been evidenced by the reported R^2, low standard deviation, and low %CV. The NanoDrop One/One^C Spectrophotometer and the associated Custom Method provide a simple and reliable procedure for quantifying endotoxin in a protein or nucleic acid sample.

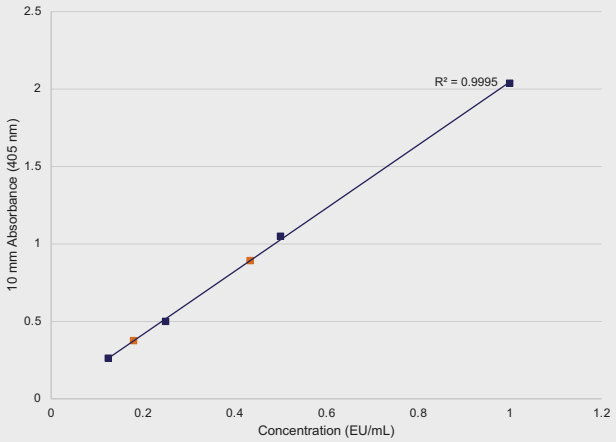

Figure 2: Endotoxin standard curve displays linearity with R^2 = 0.9995. Absorbance was measured at 405 nm using the NanoDrop One/One^C Spectrophotometer. Standard samples are shown as dark blue squares and unknown samples are shown as orange squares.

	Average A405	Standard deviation	%CV
Sample 1	0.893	0.009	1.03
Sample 2	0.376	0.011	2.80

Table 1: Unknown average sample absorbance, standard deviation, and %CV calculated from triplicate measurements using the Endotoxin Custom Method on the NanoDrop One/One^C Spectrophotometer.

NanoDrop One/One^C Spectrophotometers

References

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