ThermoFisher SCIENTIFIC

Powerfully simple digital PCR

Digital PCR

Assessment of using the QuantStudio Absolute Q Digital PCR System to quantify residual *E. coli* DNA

A potential application in gene therapy development and manufacturing processes

Abstract

E. coli host cells are commonly used to produce various therapeutic biologics. Quantification of residual DNA as an impurity in gene therapy development and manufacturing processes using quantitative PCR (qPCR) has been a widely adopted method. In recent years, digital PCR (dPCR) has gained popularity based on its higher sensitivity and independence of running standard curves, compared with qPCR. This study explored the potential of running the Applied Biosystems™ resDNASEQ™ Quantitative *E. coli* DNA Kit (Cat. No. 4458435) on the Applied Biosystems™ QuantStudio™ Absolute Q™ Digital PCR System, with the aim of providing a residual DNA quantification solution for dPCR users.

applied biosystems

Introduction

qPCR-based residual DNA testing is an established method that is routinely used for the assessment of purity and safety of therapeutic biologics. The amount of residual DNA in final products must meet regulatory guidelines established by the World Health Organization (WHO), the European Pharmacopoeia, the US Food and Drug Administration (FDA), and other global regulatory agencies. Thermo Fisher Scientific offers the resDNASEQ *E. coli* kit as a highly sensitive off-the-shelf solution to quantify residual *E. coli* DNA throughout the downstream process of common biologics manufacturing.

Whereas qPCR is a robust and proven technology, interest in dPCR has grown significantly only recently. dPCR technology allows absolute quantification without the need for standard curves. The QuantStudio Absolute Q Digital PCR System is the newest platform in the Applied Biosystems™ PCR portfolio. It consolidates what is normally a multistep, multi-instrument workflow into a simple qPCR-like workflow (Figure 1).

This study leveraged the existing resDNASEQ *E. coli* kit by testing it on the QuantStudio Absolute Q system. The performance was evaluated against detection sensitivity, quantification accuracy, and replicate uniformity.

Figure 1. QuantStudio Absolute Q Digital PCR System workflow. A reaction is prepared with all required components for dPCR including master mix, nucleic acid, and primers and probes, and then loaded into the Applied Biosystems™ QuantStudio™ Absolute Q™ MAP16 Digital PCR Plate. The dPCR experiment is run, and data are analyzed using a single instrument.

Materials and methods

dPCR reaction preparation

The resDNASEQ *E. coli* kit includes a DNA control, 10X assay mix, and negative control (water). The kit was supplemented with the appropriate reagents and consumables to operate on the QuantStudio Absolute Q Digital PCR System, including Applied Biosystems™ Absolute Q™ DNA Digital PCR Master Mix (5X) and Applied Biosystems™ QuantStudio™ Absolute Q™ Isolation Buffer formulated for the QuantStudio Absolute Q MAP16 plates. Table 1 shows the components of the dPCR reaction mix for reaction preparation.

DNA dilution preparation

DNA control dilutions were made as suggested in the Applied Biosystems™ resDNASEQ™ Quantitative DNA Kits user guide (Pub. No. 4469836).

Plate preparation for dPCR

Table 2 shows the PCR plate layout. Selected standards (SD3, SD4, SD5, and SD6) were loaded in triplicates in the QuantStudio Absolute Q MAP16 plate and subsequently loaded onto the QuantStudio Absolute Q dPCR instrument. The thermal cycler protocol was based on the existing qPCR protocol with minor modifications (Table 3).

Table 1. Components of dPCR reaction mix and overlay.

dPCR mix	Volume (µL)
Absolute Q DNA Digital PCR Master Mix (5X)	1.8
10X assay mix	0.9
Water	1.3
DNA control	5
Total volume	9
Overlay	**Volume (µL)**
QuantStudio Absolute Q Isolation Buffer	15

Table 2. PCR plate setup.

Add 24 µL of QuantStudio Absolute Q Isolation Buffer to each unused well in the QuantStudio Absolute Q MAP16 plate.				
	1	**2**	**3**	**4**
A	SD3	SD3	SD3	NTC
B	SD4	SD4	SD4	NTC
C	SD5	SD5	SD5	NTC
D	SD6	SD6	SD6	NTC

Table 3. PCR protocol.

Steps	Temperature	Time	Cycle number
Preheat	95°C	10 min	1
Denaturing	95°C	15 sec	40
Annealing and extension	60°C	1 min	

Results

Detection sensitivity, quantification accuracy, and replicate uniformity were assessed using control DNA ranging from 30 pg to 0.03 pg (Table 4). The input by mass can be converted into copy number using our **DNA copy number calculator**. Detection sensitivity goes down to SD6, with less than 10 theoretical copies being detected. Compared to qPCR, SD6 is not typically used for standard curve calculation but to confirm limit of detection (LOD). This observation implies that the assay may have a lower LOD on the dPCR instrument. Quantification accuracy lies at 84.8–97.4% consistently among all dilutions, falling within the USP 509 guideline reference range of 50–150%. Replicate uniformity is stringent at SD3, SD4, and SD5, with coefficient of variation (CV) less than or close to 15%, compared with USP 509 guideline reference CV of 30%.

Table 4. dPCR results.

Sample name	Theoretical sample input mass (pg)	Calculated copy number	Mean of measured copy number	Assay accuracy (measured/theoretical); reference: 70–130%*	CV (among 3 replicates); reference: 30%
SD3	30	5,910	5,026.68	85.1%	3.28%
SD4	3	591	528.57	89.4%	3.15%
SD5	0.3	59.1	50.13	84.8%	16.14%
SD6	0.03	5.91	5.76	97.4%	36.21%
NTC	0	0	0	100%	NA

* USP 509 guideline refers to a range of 50–150%; this study sets a tighter range.

Conclusion

The study explored the performance of the resDNASEQ *E. coli* kit on the QuantStudio Absolute Q Digital PCR System. This initial study demonstrated great potential that the QuantStudio Absolute Q system can be used to perform *E. coli* residual DNA quantification for therapeutic biologics impurity assessment.

Learn more at **thermofisher.com/absoluteq**

For Research Use Only. Not for use in diagnostic procedures. © 2022 Thermo Fisher Scientific Inc. All rights reserved.
All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. **COL26979 1122**