Technical Report

Testing for Contaminants in Rainin Tips

High Sensitivity Analysis

Although numerous factors can adversely affect molecular biology procedures, the most significant contaminants are DNA, DNase, and RNase. To make sure that common reactions such as PCR and qPCR are not affected by these factors, Rainin regularly performs several tests on each lot of the tips it produces.

Materials and methods

For the following tests a tip eluate was prepared. Ten tips from each lot of tips were exposed to 1000 µL of molecular biology grade water. The maximum volume of the tip was aspirated and dispensed two times to ensure that any contamination, it would be released.

DNA detection: Deoxyribonucleic acid (DNA) is integral to most molecular biology applications, and any exogenous DNA can significantly alter experimental outcome. Quantitative PCR (qPCR) was utilized as follows: an ABI 7700 System and Power SYBR Green master mix (ABI) were used in 25 µL reactions containing negative controls, varying concentrations of stock DNA (human or bacterial) and tip eluate. Final primer concentration was 250 nM per reaction. Both human and bacterial primer sets for conserved sequences were used as follows:

Human primers:
Forward: 5′-AAGTGTCAAGGCCAGGAGTTTG
Reverse: 5′-TCCTTCAGCTGGGCTCTCTTAC

Bacterial primers:
Forward: 5′-CCAGCAGCCGCGGTAAT
Reverse: 5′-TGCGCTTTACGCCCAGTAAT

All reactions were performed in triplicate. Ct values were analyzed to ensure that the Rainin tip eluate was below the 1 pg level.

DNase detection: Deoxyribonuclease (DNase) is an enzyme that degrades DNA. 10 µL each of negative control, sample exposed to tips, and low-threshold standard of 1 x 10^{-7} Kunitz units of DNase were incubated with a stock DNA/buffer solution for 1 hour at 37° C. Samples were run out on a 0.8% agarose gel, separated via electrophoresis, and observed via ethidium bromide staining and transilluminator. Results were confirmed, via imaging software, that DNA exposed to the tip eluate is not degraded by comparison to the negative and positive controls.

RNase detection: Ribonuclease (RNase) is a robust enzyme that degrades RNA readily, and is one of the most dangerous potential contaminants for qRT-PCR procedures. 10 µL each of negative control, sample exposed to tips, and low-threshold standard of 1x10^{-9} Kunitz units of RNase A were incubated with a stock RNA/buffer solution for 1 hour at 37° C. Samples were run out on a 1.0% agarose gel, separated via electrophoresis, and observed via ethidium bromide staining and transilluminator. Results were confirmed, via imaging software, that DNA exposed to the tip eluate is not degraded by comparison to the negative and positive controls.

METTLER TOLEDO

Results

DNA

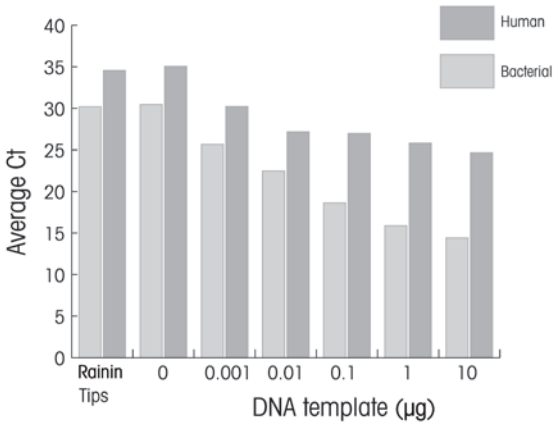

Figure 1.
qPCR data showing standard curve and test samples.
Standard curve shown from zero to 10 µg.
Rainin test samples show DNA levels below 1 pg, indicating less than 1 copy number of human DNA template contamination.

RNase

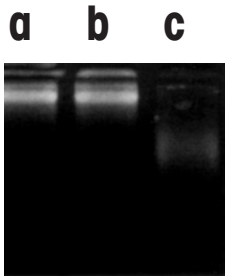

Figure 2.
a: Product samples
b: Unexposed RNA standard as a negative control
c: RNA standard exposed to RNase as a positive control

DNase

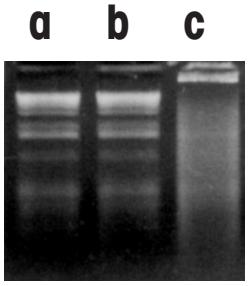

Figure 3.
a: Product samples
b: Unexposed DNA standard as a negative control
c: DNA standard exposed to DNase as a positive control

Conclusions

qPCR is a powerful amplification and detection technology that can be used for very high sensitivity analysis of genomic samples. In this series of experiments, Rainin has demonstrated the effective use of qPCR as a quantitative detection tool to verify that tips manufactured and packaged in the Rainin facility are ultra clean. Results show that Rainin can certify BioClean tips down to less than 1 copy number of human DNA template. Additionally, the most sensitive gel-based assays reveal that Rainin tips are free from RNase and DNase contamination.

Mettler-Toledo Rainin, LLC
7500 Edgewater Drive, Oakland, CA 94621
Phone +1 510 564 1600
Fax +1 510 564 1604

www.mt.com/rainin
For more information

Printed on demand from online content. TR-618 Rev B