# Preserving DNA integrity with Low DNA Binding Snap Cap Microcentrifuge Tubes

## Introduction

Quantitative PCR (qPCR) is an essential and highly versatile molecular biology technique with applications ranging from gene expression analysis to human identification in forensics workflows. Unlike endpoint measurement in conventional PCR, qPCR is performed with fluorescent reporter dyes that enable DNA quantitation in each amplification cycle. qPCR also has a wider dynamic range than conventional PCR, spanning from ~$10^{11}$ copies of genomic DNA (gDNA) down to a single copy per reaction.

A typical qPCR run includes calibration standards with known DNA concentrations that span the quantitative range of the assay. At the end of the run, the qPCR system software generates a standard curve by plotting the threshold cycle ($C_t$) against $\log_{10}$ of the corresponding DNA concentration (copies/µL) for each calibration standard. DNA in samples can then be quantified based on the experimentally determined $C_t$ values, and amplification efficiency can be estimated from the slope of the best fit line using Equation 1.

**Equation 1** Efficiency = $[10^{(-1/\text{slope})}] - 1$

Using reliable standards is critical for accurate quantitation and interpretation of amplification efficiency, although this can be difficult or impossible if standards are prepared or stored in tubes that adhere to DNA. DNA adhesion (binding) can be especially problematic at the low end of the quantitative range, where the target concentration may be as low as 10 copies/µL. Loss due to adhesion cannot be ignored at such low concentrations, because the error will be propagated in every qPCR cycle.

**The number of amplicons per reaction should theoretically double in every qPCR cycle assuming 100% amplification efficiency. However, the amplification efficiency will appear lower than it actually is if the effective DNA concentration in the standards is reduced by adhesion.**

Minimizing the risk of human DNA contamination is also important, particularly in forensics workflows. Forensics laboratories often work with samples that contain very little target DNA. Fluorescence from amplification of human DNA contaminants during qPCR can overwhelm fluorescence from amplification of the target DNA and completely invalidate a forensic analysis. Forensics laboratories rely on manufacturers of tubes and other consumables to provide a consistent supply of high-quality products with minimal risk of human DNA contamination from manufacturing processes.

This application note describes a performance evaluation of the Low DNA Binding Snap Cap Microcentrifuge Tubes (MCTs offered by Thermo Fisher Scientific). These MCTs were assessed for DNA binding and human DNA contamination, and their binding performance was compared to that of MCTs from six other suppliers.

**Thermo Scientific™ Low DNA Binding Snap Cap Microcentrifuge Tubes and Thermo Scientific™ Low DNA Binding Snap Cap Microcentrifuge Tubes, Sustain™ Series were developed to preserve nucleic acid integrity for research, pharmaceutical, and forensics applications.**

## Materials and methods

### DNA binding experiment

A human gDNA dilution series was prepared using Applied Biosystems™ TaqMan™ Control Genomic DNA (Cat. No. 4312660) and Invitrogen™ Nuclease-Free Water (Cat. No. AM9930) (Table 1). Each solution was vortexed for 1 minute before preparing the next dilution. A no-template control (NTC) was also prepared using only nuclease-free water.

**Table 1. Preparation of gDNA dilution series for DNA binding experiments.**

| Volume of gDNA added | Water (µL) | Final gDNA concentration (pg/µL) | Concentration identifier | gDNA copies per 9 µL* |
|---|---|---|---|---|
| 5.5 µL from 10 ng/µL stock | 1,494.5 | 36.7 | DNA-3 | 100 |
| 150 µL from DNA-3 | 1,350 | 3.67 | DNA-2 | 10 |
| 150 µL from DNA-2 | 1,350 | 0.37 | DNA-1 | 1 |
| 0 | 1,350 | 0 | DNA-0 | 0 |

* The dilution scheme was based on the number of gDNA copies per 9 µL, because 9 µL would be used to quantify the gDNA by qPCR the next day. One copy of human gDNA = 3.3 pg.

### Labeling and organization of MCTs for overnight storage at –80°C

Low DNA Binding Snap Cap 1.5 mL MCTs and low-binding 1.5 mL MCTs from the other suppliers were labeled with the concentration identifiers shown in Table 1. A test replicate consisted of four MCTs from the same supplier labeled DNA-0, DNA-1, DNA-2, and DNA-3. The MCTs were grouped into test replicates according to supplier in a storage rack, and 55 µL diluted gDNA was aliquoted into each MCT labeled with the corresponding concentration identifier.

The MCTs were stored at –80°C for 24 hours, and the process was repeated on subsequent days for a total of three racks of MCTs. The total number of replicates prepared to obtain a statistically valid sample size is shown in Table 2. A minimum of nine replicates were prepared per supplier. Additional replicates were prepared with MCTs from Suppliers A, C, and F to increase the size of the dataset, although this ultimately had little impact on the analysis.

**Table 2. Test replicates stored overnight at –80°C for the DNA binding experiment.**

| MCTs by supplier | Number of replicates |
|---|---|
| Low DNA Binding Snap Cap MCTs | 18 |
| Supplier A MCTs | 12 |
| Supplier B MCTs | 9 |
| Supplier C MCTs | 12 |
| Supplier D MCTs | 9 |
| Supplier E MCTs | 9 |
| Supplier F MCTs | 12 |

### qPCR

Quantitative PCR was performed to quantify any loss of gDNA due to adhesion to the MCTs during storage at –80°C. Aliquots were transferred from the MCTs to individual wells in 12-well strip tubes to facilitate multichannel pipetting for qPCR. A fresh gDNA dilution series was also prepared in a popular line of low-binding MCTs on the day of qPCR as outlined in Table 1. Aliquots from these tubes served as positive qPCR controls.

In a 15 mL conical tube, 3.60 mL Applied Biosystems™ TaqMan™ Universal Master Mix II, no UNG (Cat. No. 4440043) was mixed with 230 µL of an Applied Biosystems™ TaqMan™ single-tube human RNase P assay (Cat. No. 4316831). A qPCR plate was prepared by aliquoting 11 µL of this mixture into individual wells along with either 9 µL from an MCT that had been stored at –80°C or 9 µL freshly prepared gDNA. Reactions labeled DNA-1, DNA-2, or DNA-3 contained 1, 10, or 100 copies of gDNA, respectively (Table 1). qPCR was run on the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System using the cycling parameters shown in Table 3.

**Table 3. qPCR cycling parameters for the DNA binding experiment.**

| Temperature | Time | Cycles |
|---|---|---|
| 50°C | 2 min | 1 |
| 95°C | 10 min | 1 |
| 95°C | 15 s | 40 |
| 60°C | 1 min | |

qPCR was performed on subsequent days with aliquots from MCTs in the other two racks stored overnight at –80°C. A fresh gDNA dilution series was prepared each day, and aliquots from those tubes served as positive controls.

## Data analysis

An average $C_t$ was calculated at each gDNA concentration for MCTs from each supplier and plotted against $\log_{10}$ of the gDNA concentration. The LINEST function in Microsoft™ Excel™ was used to obtain best fit line statistics for MCTs from each supplier, and qPCR amplification efficiency was calculated using Equation 1. Propagated error was also calculated for MCTs from each supplier by multiplying the respective amplification efficiency by ln(10) times the error in the slope.

## Assay for human DNA contamination

Low DNA Binding Snap Cap MCTs (n = 180) from 18 different lots were tested for human DNA contamination. The MCTs were placed in one of three bags designated by MCT volume (0.6 mL, 1.5 mL, or 2.0 mL). MCTs in the same bag were removed at random and organized into six groups of ten MCTs each. A 100 µL aliquot of nuclease-free water was added to one MCT in each group. The MCT was vortexed for 1 minute, and the aliquot was transferred to another MCT in the same group. This process was repeated until all 10 MCTs in the group had been vortexed with the same 100 µL aliquot of water, and the last MCT containing the aliquot was incubated at 90°C for 10 minutes.

A third-party laboratory performed qPCR to test the aliquots for human DNA contamination. Reactions were prepared with Applied Biosystems™ AmpliTaq Gold™ DNA Polymerase with Buffer II and $MgCl_2$ (Cat. No. N8080249). Human buccal DNA was used to prepare positive control samples, and negative controls were prepared with PCR-grade water. qPCR was run for 32 cycles with individual wells containing 30 µL each. The qPCR products were then analyzed by gel electrophoresis to determine whether human DNA contamination was present. The detection limit of the assay was 1 pg DNA (0.03 pg/µL), and the criterion for passing was <5 pg human DNA.

# Results

## DNA binding experiment

qPCR amplification efficiency ranged from 103% to ~115% across suppliers (Table 4). qPCR amplification efficiency with aliquots stored in Low DNA Binding Snap Cap MCTs was in the middle of this range at 108.2%. The associated propagated error was 24.1%, which again fell near the middle of the range from 1.1% to 59.2%. These results show that Low DNA Binding Snap Cap MCTs can be used for short-term –80°C storage of human gDNA qPCR standards down to one copy per reaction without any significant loss due to adhesion.

**Table 4. Results of qPCR to evaluate gDNA loss due to adhesion to MCTs during storage at –80°C.**

| MCTs | Average $C_t$ | | | $R^2$ | Slope | qPCR efficiency | Propagated error |
|---|---|---|---|---|---|---|---|
| | DNA-1 | DNA-2 | DNA-3 | | | | |
| Positive control MCTs | 37.2 | 33.8 | 30.7 | 0.9994 | –3.25 | 103.3% | 19.5% |
| Low DNA Binding Snap Cap MCTs | 37.1 | 34.1 | 30.8 | 0.9991 | –3.14 | 108.2% | 24.1% |
| Supplier A MCTs | 37.0 | 34.2 | 30.8 | 0.9954 | –3.12 | 109.3% | 53.2% |
| Supplier B MCTs | 36.9 | 34.3 | 30.9 | 0.9945 | –3.02 | 114.5% | 59.2% |
| Supplier C MCTs | 37.2 | 34.1 | 30.8 | 0.9996 | –3.21 | 105.0% | 16.0% |
| Supplier D MCTs | 37.1 | 34.0 | 30.8 | 1 | –3.17 | 106.9% | 1.1% |
| Supplier E MCTs | 37.0 | 34.1 | 30.8 | 0.9994 | –3.12 | 109.0% | 19.3% |
| Supplier F MCTs | 37.4 | 34.2 | 30.9 | 0.9999 | –3.24 | 103.7% | 6.8% |

## Assay for human DNA contamination

The results of the assay supported the conclusion that all Low DNA Binding Snap Cap MCTs met the criterion of <5 pg human DNA. A representative electrophoresis gel is shown in Figure 1.

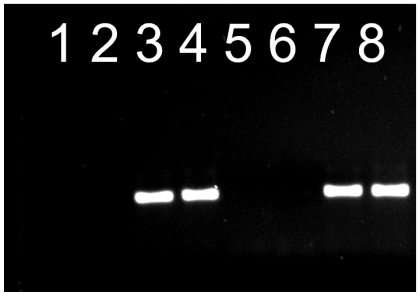


**Figure 1. Representative electrophoresis gel from the human DNA contamination assay.** Lanes 5 and 6 were loaded with MCT test samples. As expected, no human DNA was detected. The samples loaded into Lanes 7 and 8 were included to test for PCR inhibition. Bands of the expected size and intensity were detected, indicating that no PCR inhibition occurred. Lanes 1 and 2: negative controls. Lanes 3 and 4: positive controls.

## Conclusion

Short-term storage of human gDNA at –80°C in Low DNA Binding Snap Cap MCTs had no significant impact on qPCR efficiency, even at very low gDNA concentrations. Low DNA Binding Snap Cap MCTs also met the criterion of <5 pg human DNA in an assay for human DNA contamination. The results of this study demonstrate that Low DNA Binding Snap Cap Microcentrifuge Tubes perform as well as or better than other low-binding MCTs on the market. They are suitable for use in sensitive forensics workflows that require accurate qPCR standards and MCTs that are free of human DNA contaminants.

Learn more at **thermofisher.com/low-dna-binding-tube**