Protein binding

Thermo Scientific microcentrifuge tubes feature low protein-binding characteristics that do not affect enzyme activity

Introduction

Importance of low protein binding

Protein binding to microcentrifuge tubes is a critical issue in molecular biology applications where the quantity of the stored protein sample is a key factor in downstream applications. It has been reported previously that due to the amphipathic nature of most proteins, they can adsorb to many surfaces [1]. When quantification from a biological sample is the endpoint of the diagnostic analysis, results or conclusions may be erroneous due to adsorption of the proteins to surfaces of labware used during the workflow. To mitigate this issue, several approaches have been suggested. For example, surface binding of proteins can be reduced by keeping samples in high-salt concentrations or by adding Tween™ 20 surfactant to samples [2]. Adding bovine serum albumin (BSA) to buffers [3] or coating surfaces with BSA [4] have also been suggested as simple solutions to the challenge of reducing protein binding to surfaces—although using BSA is not always an option, particularly in mass spectrometry applications. Another approach has been to identify the surfaces used during laboratory applications, such as glass and plastic, and choose an option that has low protein-binding properties [5].

In the experiments described here, we investigated the influence of storing *Taq* polymerase in polypropylene microcentrifuge tubes manufactured by Thermo Fisher Scientific or Sarstedt on polymerase activity, as measured by qPCR and fluorescence resonance energy transfer (FRET). The results from qPCR and FRET show no difference between the two brands of tubes in polymerase activity, indicating that for this enzyme, both tube brands exhibited low protein-binding properties.

Materials and methods

Aliquots of 100 µL of **Applied Biosystems™ AmpliTaq Gold™ 360 DNA Polymerase (Cat. No. 4398823)** were stored for 4 weeks at –20°C in either 1.5 mL clear Thermo Scientific™ tubes or 1.5 mL clear Sarstedt™ tubes. The aliquots of polymerase were used in both qPCR and FRET assays to measure the enzyme (polymerase) activity after being stored, to determine whether or not the protein is binding to the microcentrifuge tubes. The polymerase activity was assessed by a qPCR assay after 3 weeks of storage and by the FRET assay at the ends of the first, second, third, and fourth weeks of storage. The stability of the enzyme is 6 years at –20°C, so polymerase degradation was not expected in these experiments.

qPCR functional assay

The PCR reaction mixes containing the polymerase that was stored in the Thermo Scientific or Sarstedt microcentrifuge tubes, and the control PCR mix containing the polymerase from the original stock, were prepared in low-binding **Invitrogen™ RNase-Free Microfuge Tubes (Cat. No. AM12450)**. Human DNA (hDNA) was diluted to 0.0001, 0.001, 0.01, 0.1, 1.0, and 10 ng/µL prior to being used as a template in the PCR. A 25 µL PCR reaction contained 0.125 µL of AmpliTaq Gold 360 DNA Polymerase from one of the storage conditions, 14.825 µL of PCR-grade water, 2.5 µL of **Applied Biosystems™ AmpliTaq™ 360 Buffer (Cat. No. 4398848)**, 1.8 µL of 25 mM $MgCl_2$, 2 µL of dNTP mix, 1.25 µL of Applied Biosystems™ Human ACTB (Beta Actin) Endogenous Control (VIC™/TAMRA™ probe, primer limited, Cat. No. 4310881E), and 2.5 µL of diluted hDNA. All samples were run in quadruplicate on a 96-well PCR plate and cycled in an **Applied Biosystems™ ViiA™ 7 Real-Time PCR System with 96-Well Block (Cat. No. 4453534)**. The differences between the means of the C_t for Thermo Scientific and Sarstedt brand tubes at each dilution of polymerase were analyzed using a Student's t-test at a significance of $\alpha = 0.05$.

Fluorescence resonance energy transfer (FRET) test

The polymerase activity was also measured by a FRET method [6] based on measuring the light generated when two fluorophores are brought into proximity during hybridization. At time 0, after the polymerase was added to the tubes, the FRET assay was used to determine the enzyme's activity. This result was then used for comparison to the enzyme's activity measured at the end of the first, second, third, and fourth week of enzyme storage. At each testing point, 10 µL of the enzyme from both the Thermo Scientific and Sarstedt tubes was serially diluted with enzyme dilution buffer up to a 2,400-fold dilution (Table 1) in low-binding surface tubes (RNase-Free Microfuge Tubes, Cat. No. AM12450), followed by transferring the dilutions to a 96-well PCR plate, which was then heated to 60°C in an Applied Biosystems™ 7500 Fast Real-Time PCR System (Cat. No. 4365464). The kinetic extension reaction was monitored for 60 isothermal cycles of 30 seconds each for 30 minutes total. The 96-well format allowed for the analysis of duplicate samples from each of the three dilutions of the polymerase that were prepared from one of two tubes from each manufacturer (Thermo Fisher and Sarstedt), as well as duplicate samples of the three dilutions of the polymerase prepared from two aliquots of the polymerase taken from the original vial. Thus, each stored enzyme sample and the control were analyzed six times (three dilutions prepared in duplicate). The results presented per tube type were averaged across enzyme dilutions. The difference between C_t means for Thermo Scientific and Sarstedt tubes for each week stored, as well as the C_t differences between weeks for each brand, were analyzed using a Student's t-test at a significance of $\alpha = 0.05$.

Table 1. Serial dilution of the original polymerase stock for the FRET test.

	Dilution A	Dilution B	Dilution C
5 U/µL	10 µL	10 µL of A	100 µL of B
Dilution buffer	190 µL	290 µL	300 µL
Dilution factor	20	600	2,400

Results

qPCR data

The C_t values from the qPCR assay are presented in Figure 1.

There is no difference in C_t values among the Thermo Scientific, Sarstedt, and control microcentrifuge tubes, indicating that storing *Taq* polymerase in microcentrifuge tubes up to 3 weeks has no effect on its functionality and indicates that the quantity of polymerase remained the same, suggesting the polymerase did not adsorb to the tube walls.

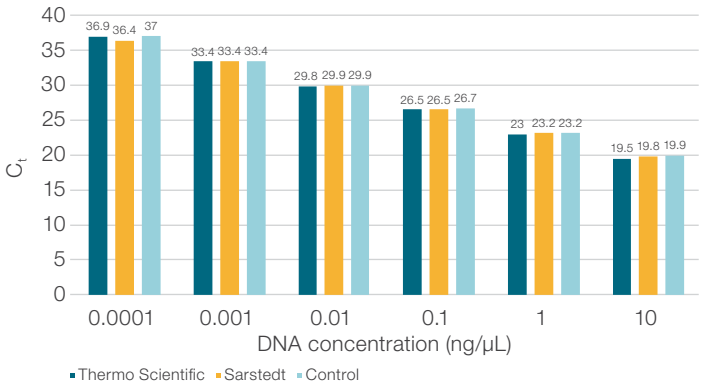

Figure 1. Average C_t values (n = 4) obtained from qPCR prepared from a fresh master mix (control) or stored for 3 weeks (Thermo Scientific and Sarstedt tubes) using template DNA concentrations of 0.0001, 0.001, 0.01, 0.1, 1.0, and 10 ng/µL.

FRET test data

Figure 2 presents the results of the FRET test for measuring the polymerase activity each week of storage in Sarstedt and Thermo Scientific tubes for a total of 4 weeks.

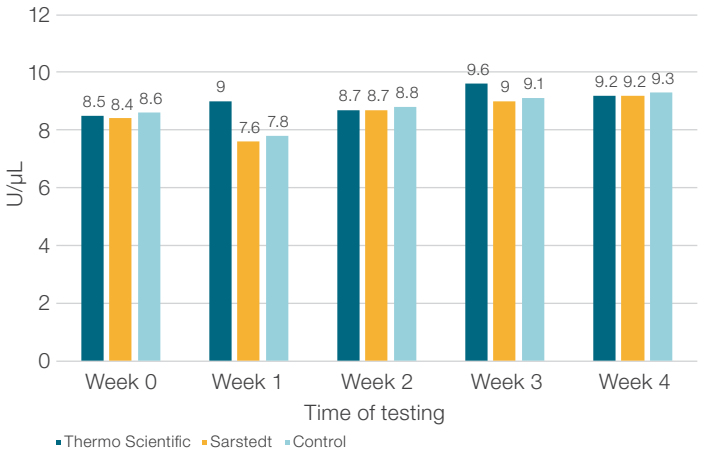

Figure 2. Polymerase activity of the enzyme after being stored for 4 weeks at –20°C in Thermo Scientific, Sarstedt, or control tubes, measured in U/µL.

There was no difference between the enzyme activities of the polymerase stored in the Sarstedt and Thermo Scientific microcentrifuge tubes. Also, there was no difference in enzyme activity between weeks of storage, indicating that storage time had no effect on the activity of the polymerase. Therefore, Sarstedt microcentrifuge tubes and Thermo Scientific microcentrifuge tubes have identical polymerase-binding characteristics.

Also, the activities of the polymerase measured by the FRET assay confirmed the results obtained by qPCR (Figure 1). There was no decrease in polymerase activity after being stored in either Sarstedt or Thermo Scientific tubes over a period of 4 weeks (Figure 2). These results confirm that there is no polymerase binding to the tubes.

Summary

There was no effect on the qPCR performance of the polymerase after being stored in either Thermo Scientific or Sarstedt microcentrifuge tubes, indicating the polymerase did not adsorb to the microcentrifuge tubes.

FRET results indicated no difference in the polymerase activity for the enzyme stored in either Thermo Scientific or Sarstedt tubes.

The amount of time the polymerase was stored in microcentrifuge tubes had no effect on the activity of the polymerase.

References

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