Fast and efficient enzyme removal with QIAquick™ Spin Kits

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Enzyme contamination of DNA samples can interfere with subsequent downstream applications. In this study, we show how QIAquick™ Spin Kits can be used for fast, easy, and highly efficient removal of a broad spectrum of enzymes widely used in molecular biology.

Materials and methods

To examine the efficiency of enzyme removal using QIAquick Kits, pure DNA samples were spiked with various enzymes and cleaned up using QIAquick Kits. The level of enzyme activity remaining in the eluates was determined according to the appropriate enzyme assays as described below (see flow chart).

Experimental procedure

Pure DNA sample
↓
Addition of various enzymes
↓
Purification using QIAquick
↓
Determination of level of remaining enzyme in eluates

Enzyme assays

Calf intestinal alkaline phosphatase (CIAP): 30 µl of a solution containing CIAP (Life Technologies) was mixed with 470 µl water and 500 µl 0.5% 4-nitrophenylphosphate (PNPP) solution, and incubated for 5 or 15 min at room temperature. The amount of 4-nitrophenol generated by hydrolysis of PNPP was determined by measurement of the absorbance at 410 nm (Beckman DU7400 spectrophotometer).

DNase I: 10 µg pUC19 plasmid DNA was incubated for 24 h at 37°C with various amounts of DNase I (Boehringer Mannheim) in a 20-µl reaction volume of 1x reaction buffer with a final concentration of 20 mM Tris·Cl, pH 8.4, 50 mM KCl, and 2 mM $MgCl_2$. The enzymatic reactions were stopped by the addition of 1 µl 0.5 M EDTA. Aliquots containing 500 ng DNA were analyzed on a 1% TAE agarose gel.

***Taq* DNA polymerase:** 2-µl aliquots of a solution containing *Taq* DNA Polymerase (QIAGEN) were pipetted onto a nylon membrane and air-dried. The filter was blocked with 10% (w/v) milk powder/TBS (10 mM Tris·Cl, 150 mM NaCl, pH 7.5) mixture for 1 h at room temperature. The first antibody reaction was performed with a 1/1000 dilution of monoclonal antibody TaqStart™ (CLONTECH) in 10% (w/v) milk powder/TBS, and the second antibody reaction was performed using an anti-mouse-POD antibody (Jackson Immunoresearch) under the same conditions. The filter was subsequently treated with a 1:1 dilution of ECL reagent (Amersham) and the chemiluminescence *Taq* DNA Polymerase activity was detected by autoradiography of the dot blot.

Alkaline Phosphatase

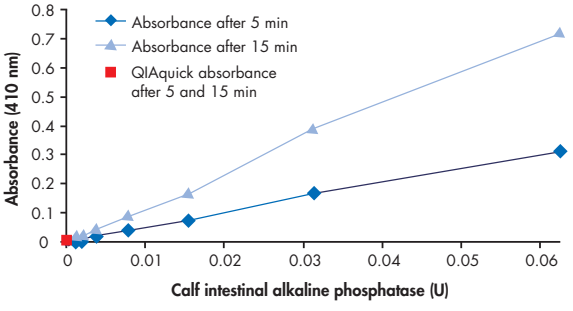

Figure 1 *The standard dilution series of calf intestinal alkaline phosphatase used to determine CIAP removal from DNA samples using QIAquick Spin Kits. Absorbance measurements were taken at 5 and 15 min. Note that the samples purified using QIAquick Spin Kits gave zero absorbance at 410 nm, indicating efficient removal of CIAP.*

T4 polynucleotide kinase: reactions were performed in a 50-µl volume containing 25 pmol of 111-base oligonucleotide, 5 µCi [γ-^{32}ATP], different amounts of T4 polynucleotide kinase (Life Technologies), and 1x reaction buffer with a final concentration of 10 mM Tris-acetate, 10 mM $MgCl_2$, 50 mM KAc, and 50 µg/ml BSA. Samples were incubated for 1 h at 37°C, after which the enzymatic reactions were stopped by heating the samples to 65°C for 10 min. 2 µl was separated on a 10% polyacrylamide gel containing 8 M urea and the level of enzymatic activity was detected by autoradiography.

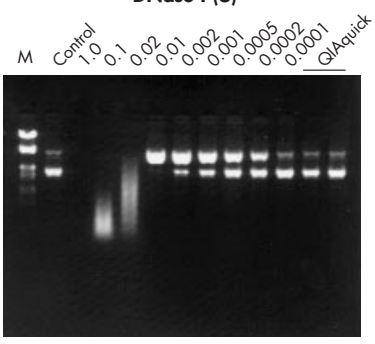

Figure 2 *Efficient DNase I removal using QIAquick Spin Kits. Lanes containing the indicated amounts of DNase I were compared with two replicate samples containing 500 ng pUC19 DNA contaminated with 0.5 U DNase I which were subsequently purified using the QIAquick PCR Purification Kit.* ***M****: lambda* EcoRI/HindIII; ***Control****: undigested pUC19.*

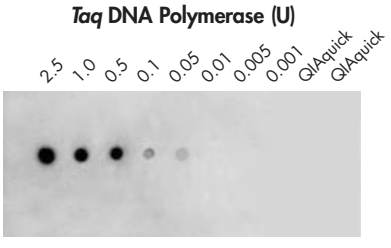

Figure 3 *Efficient* Taq *DNA Polymerase removal using QIAquick Spin Kits. Lanes containing the indicated amounts of* Taq *DNA Polymerase were compared with two replicate samples containing 100 ng linearized pUC19 DNA contaminated with 2.5 U* Taq *DNA Polymerase which were subsequently purified using the QIAquick PCR Purification Kit.*

Klenow fragment: reactions were performed in a 20-µl reaction volume containing 400 ng 125-bp DNA (with sticky ends), various amounts of Klenow fragment (Life Technologies), nucleotides at final concentrations of 25 µM (dATP, dTTP, dGTP, and [α–^{32}dCTP]) in 1x reaction buffer for 16 h at 37°C. 4 µl aliquots were separated on a 6% polyacrylamide gel and Klenow activity was detected by autoradiography.

DNA spiking with enzymes, purification with QIAquick Spin Kits, and enzyme assays

Pure DNA samples were spiked with various amounts of enzyme as follows: for CIAP, 500 ng linearized pUC21 DNA was mixed with 0.5 U CIAP; for DNase I, 10 µg pUC19 plasmid DNA was mixed with 0.5 U DNase I; for *Taq* DNA Polymerase, 100 ng linearized pUC19 DNA was mixed with 2.5 U *Taq* DNA Polymerase; for T4 polynucleotide kinase, 25 pmol of 111-base oligonucleotide was mixed with 2.5 U T4 polynucleotide kinase; for Klenow fragment, 400 ng of 125-bp DNA was mixed with 3.3 U Klenow fragment. All contaminated samples were then purified using the QIAquick PCR Purification Kit, the QIAquick Nucleotide Removal Kit, or the QIAquick Gel Extraction Kit*. The eluates were adjusted to the appropriate assay conditions for enzymatic activity, and the levels of enzyme removal were determined.

Results

All QIAquick Spin Kits gave the same high level of enzyme removal. To illustrate this, we have presented the results obtained with the QIAquick PCR Purification Kit. Figure 1 shows the standard calf intestinal alkaline phosphatase dilution curve used to determine the level of CIAP removal. Using this sensitive assay for CIAP detection, the samples purified using QIAquick Spin Kits gave zero absorption at 410 nm, indicating efficient CIAP removal to <0.0004 U. The ability of QIAquick Spin Kits to remove DNase I to <0.0002 U is shown in Figure 2. Figure 3 shows the effective removal of *Taq* DNA Polymerase to <0.05 U, and T4 polynucleotide kinase can be removed to <0.025 U (Figure 4). QIAquick Spin Kits removed Klenow fragment to <0.01 U (Figure 5).

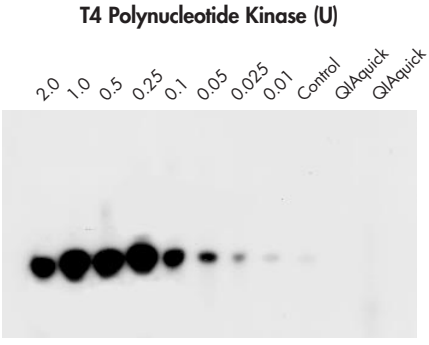

Figure 4 *Efficient T4 polynucleotide kinase removal using QIAquick Spin Kits. Lanes containing the indicated amounts of T4 polynucleotide kinase were compared to two replicate samples containing 25 pmol of 111-base oligonucleotide contaminated with 2.5 U T4 polynucleotide kinase which were subsequently purified using the QIAquick PCR Purification Kit.* ***Control****: 25 pmol of 111-base oligonucleotide plus 5 µCi [γ–^{32}ATP].*

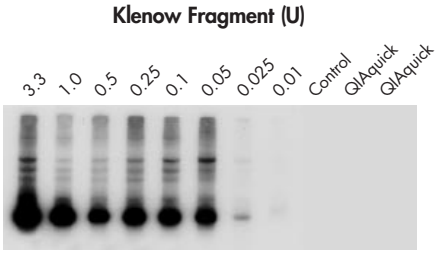

Figure 5 *Efficient Klenow fragment removal using QIAquick Spin Kits. Lanes containing the indicated amounts of Klenow fragment were compared to two replicate samples containing 400 ng of 125-bp DNA contaminated with 3.3 U Klenow fragment which were subsequently purified using the QIAquick PCR Purification Kit.* ***Control****: nucleotides at final concentrations of 25 µM (dATP, dTTP, dGTP, and [α–^{32}dCTP]), plus 400 ng of 125-bp fragment.*

** Note that it is not necessary to run enzymatic reactions on a gel prior to cleanup with the QIAquick Gel Extraction Kit. Simply add 3 volumes binding buffer QG to 1 volume enzymatic reaction (omitting the heating step) and follow the QIAquick Gel Extraction Kit Protocol in the* QIAquick Spin Handbook *starting at step 5 (100 mg gel ≅ 100 µl).*

Conclusions

QIAquick Spin Kits enable efficient and reliable removal of a broad range of enzymes such as calf intestinal alkaline phosphatase from DNA samples. In addition to the enzymes tested above, these kits also effectively remove many of the other enzymes commonly used in molecular biology (Table 1; see also page 8 of the *QIAquick Spin Handbook* for detailed specifications). The efficiency, versatility, and convenience of QIAquick Spin Kits make them the ideal choice to clean up DNA after various enzymatic reactions.

We have recently created a colorful QIAquick Selector Wheel Poster indicating the many enzymatic reactions that can be cleaned up using QIAquick Kits. To order your poster, turn to the BRC in the center of this issue of *QIAGEN News*. ■

Table 1. The QIAquick Kits suitable for cleanup of various enzymatic reactions

	QIAquick PCR Purification Kit	QIAquick Nucleotide Removal Kit	QIAquick Gel Extraction Kit*
Alkaline phosphatase	**YES**	**YES**	**YES**
cDNA synthesis	**YES**	no	no
DNase, nuclease digestion	**YES**	**YES**	**YES**
Kinase:			
DNA fragments	**YES**†	**YES**	**YES**†
oligonucleotides	no	**YES**	no
Ligation	**YES**	**YES**	**YES**
Nick translation	**YES**†	**YES**	**YES**†
PCR	**YES**	no	no
Random priming	**YES**†	**YES**	**YES**†
Restriction	**YES**	**YES**	**YES**
Tailing:			
DNA fragments	**YES**†	**YES**	**YES**†
oligonucleotides	no	**YES**	no

** Note that it is not necessary to run enzymatic reactions on a gel prior to cleanup with the QIAquick Gel Extraction Kit. See footnote on previous page.*

† Although enzyme removal is possible with all QIAquick Kits, their different fragment-size recovery and removal specifications mean that some kits are more suitable than others for certain applications. The QIAquick Nucleotide Removal Kit provides slightly more convenient handling for these applications. Please see page 8 of the QIAquick Spin Handbook.

Ordering Information

Product	Contents	Cat. No.
QIAquick Kits*		
QIAquick PCR Purification Kit (50)	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28104
QIAquick 8 PCR Purification Kit (10)†	For purification of 10 x 8 PCR reactions: 10 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2-ml), Caps	28142
QIAquick 96 PCR Purification Kit (1)‡	For purification of 1 x 96 PCR reactions: 1 QIAquick 96 Plate, Buffers, Collection Microtubes (1.2-ml), Caps	28180
QIAquick Nucleotide Removal Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28304
QIAquick Gel Extraction Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28704

**Other kits and sizes available* *† Requires use of QIAvac 9S* *‡ Requires use of QIAvac 96*