Hints for optimum elution of DNA from QIAprep™ and QIAquick™ spin columns

Silica-gel membrane technology is a proven molecular biology method for the purification of a variety of nucleic acids. It is used in QIAprep™ Kits for plasmid DNA minipreps, and in QIAquick™ Kits for DNA cleanup from agarose gels, or PCR and other enzyme reactions. The advantage of silica-gel–membrane technology is that the DNA can be eluted in a small volume of low-salt buffer, ready to use in subsequent applications.

The elution efficiency and DNA concentration with QIAprep and QIAquick spin columns depend on four main factors: the volume of elution buffer used, the pH and temperature of the elution buffer, and the length of time the buffer is incubated on the membrane. Here are some tips to help you maximize your DNA yields.

Elution volume

A volume of 100–200 µl of elution buffer completely covers the membrane and ensures maximum yields even when not applied directly to the center of the membrane (Figures 1A and 1B). However, if a more concentrated eluate is desired, volumes as small as 50 µl for QIAprep spin columns and 30 µl for QIAquick spin columns can be used instead.* In these cases, it is important to add the buffer directly to the center of the membrane so that the membrane is completely covered.

Incubation time

For optimum DNA yields when eluting with small volumes such as 30 µl, a 1-min incubation after the addition of elution buffer is required before centrifugation, in order to allow the adsorbed DNA to go into solution. For QIAquick spin columns, the DNA will be up to 1.7 times more concentrated if the column is incubated for 1 min with 30 µl of elution buffer, than if eluted in 50 µl without incubation prior to centrifugation (Figures 1B and 2).

Note: With multiwell formats, minimum elution-buffer volumes of 75 µl and 60 µl should be used for QIAprep and QIAquick multiwell kits, respectively.

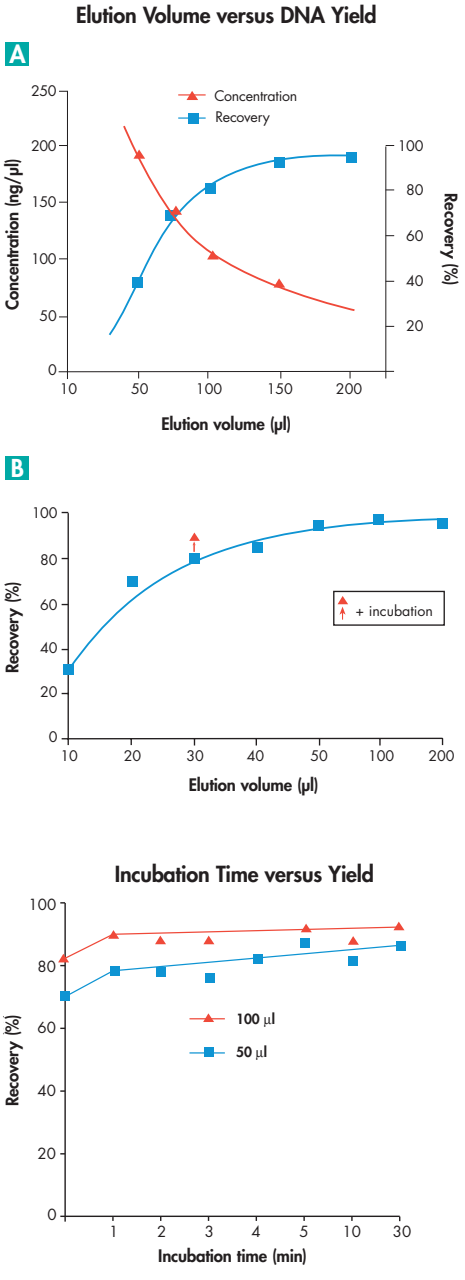

Figure 1 *Effect of elution-buffer volume on DNA yield for* **A** *the QIAprep 8 Miniprep Kit, and* **B** *the QIAquick PCR Purification and QIAquick Nucleotide Removal Kits.* **A** *10 µg pUC18 was purified using the QIAprep 8 Miniprep protocol, and eluted with the indicated volumes of Buffer EB (10 mM Tris·Cl, pH 8.5).* **B** *5 µg of a 2.9-kb DNA fragment was purified using either the QIAquick PCR Purification Kit protocol or the QIAquick Nucleotide Removal Kit protocol and eluted with the indicated volumes of Buffer EB. Note that 30 µl plus 1-min incubation on the QIAquick spin column gives DNA yields similar to 50 µl without incubation, but at a concentration of 1.7 times greater.*

Figure 2 *10 µg pBluescript® was purified using the QIAprep Spin Miniprep protocol and eluted after the indicated incubation times with Buffer EB.*

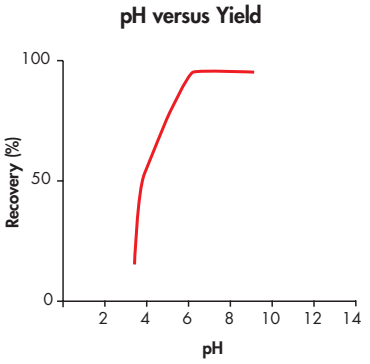

Figure 3 *pH dependence of DNA elution from silica-gel membranes. 1 µg of a 2.9-kb DNA fragment was eluted with Buffer EB at different pHs. The graph shows the percentage of DNA elution.*

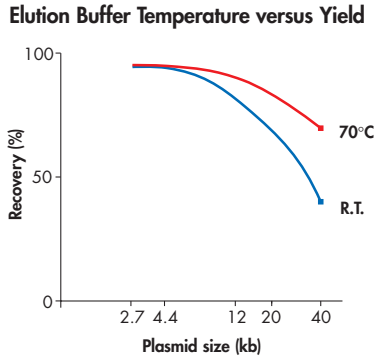

Figure 4 *Effect of elution-buffer temperature on DNA yield from silica-gel membranes. Plasmids of various sizes were either eluted with room-temperature (**R.T.**) Buffer EB, or Buffer EB preheated to 70°C.*

pH

Maximum elution efficiency from silica is obtained using low-salt buffers with a pH between 6.5 and 8.5 (Figure 3). The elution buffer EB (10 mM Tris·Cl, pH 8.5) included in QIAquick and QIAprep Kits offers both optimal salt and pH conditions. As this buffer does not contain any additional salt, the eluted DNA is suitable for subsequent use in salt-sensitive applications such as ligation or automated sequencing. When using water for elution, make sure that the pH value is within the range 7.0 to 8.5. Store DNA at –20°C when eluted in water as DNA may degrade in the absence of a buffering agent at higher temperatures.

Temperature

Room-temperature elution buffer is suitable for elution of plasmids <10 kb. Larger plasmids (>10 kb) or cosmids, are more difficult to elute from silica, so the temperature of the elution buffer should be increased to 70°C (Figure 4).

DNA concentration of sample

For QIAquick spin columns, the DNA concentration of the eluate depends on the amount of DNA in the sample to be cleaned up, as well as the volume of elution buffer used. Table 1 shows the DNA concentrations obtained following QIAquick spin and QIAquick Mulitwell cleanup of a range of PCR products. Similarly, for QIAprep spin columns, the yield of DNA depends on the DNA concentration of the miniprep (see the *QIAprep Miniprep Handbook).*

Recommendations for optimum elution

- For highest yield use the maximum elution volume possible
- For elution volumes ≤100 µl pipet buffer directly onto the center of the silica membrane
- Include a 1-min incubation prior to centrifugation when using a small elution volume
- Use a low-salt elution buffer with a neutral to slightly basic pH
- Increase the temperature of the elution buffer to 70°C when eluting large DNA fragments (>10 kb). ■

Table 1. DNA concentration after QIAquick cleanup

PCR products (µg)	DNA concentration (ng/µl) after QIAquick spin cleanup*		DNA concentration (ng/µl) after QIAquick 8/96 cleanup†	
	With 28 µl eluate	With 48 µl eluate	With 30 µl eluate	With 50 µl eluate
0.50	16	9	13	8
0.75	24	14	20	12
1.0	32	19	27	16
1.5	48	28	40	24
2.0	64	38	53	32
3.0	96	56	80	48

** Based on 90% recovery and 2 µl dead volume*
† Based on 80% recovery and 30 µl dead volume

Ordering Information

Product	Contents	Cat. No.
QIAprep Kits		
QIAprep Spin Miniprep Kit (50)	For 50 high-purity plasmid minipreps: 50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2-ml)	27104
QIAprep Spin Miniprep Kit (250)	For 250 high-purity plasmid minipreps: 250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2-ml)	27106
QIAprep 8 Miniprep Kit (50)*†	For 50 x 8 high-purity plasmid minipreps: 50 QIAprep 8 Strips, Reagents, Buffers, Collection Microtubes (1.2-ml), Caps	27144
QIAprep 8 Turbo Miniprep Kit (10)†§	For 10 x 8 high-purity plasmid minipreps, 10 each: TurboFilter 8 and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2-ml), Caps	27152
QIAprep 8 Turbo Miniprep Kit (50)†§	For 50 x 8 high-purity plasmid minipreps, 50 each: TurboFilter 8 and QIAprep 8 Strips; Reagents, Buffers, Collection Microtubes (1.2-ml), Caps	27154
QIAprep 96 Turbo Miniprep Kit (1)‡§	For 1 x 96 high-purity plasmid minipreps, 1 each: TurboFilter 96 and QIAprep 96 Plates; Flat-Bottom Block and Lid, Reagents, Buffers, Collection Microtubes (1.2-ml), Caps	27190
QIAprep 96 Turbo Miniprep Kit (4)‡§	For 4 x 96 high-purity plasmid minipreps, 4 each: TurboFilter 96 and QIAprep 96 Plates; Flat-Bottom Blocks and Lids, Reagents, Buffers, Collection Microtubes (1.2-ml), Caps	27191
QIAquick Kits		
QIAquick PCR Purification Kit (50)§¶	For purification of 50 PCR reactions: 50 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28104
QIAquick PCR Purification Kit (250)§¶	For purification of 250 PCR reactions: 250 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28106
QIAquick Nucleotide Removal Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28304
QIAquick Nucleotide Removal Kit (250)	250 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28306
QIAquick Gel Extraction Kit (50)	50 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28704
QIAquick Gel Extraction Kit (250)	250 QIAquick Spin Columns, Buffers, Collection Tubes (2-ml)	28706

** Other kit sizes available*
† Requires use of QIAvac 6S
‡ Requires use of QIAvac 96
§ Special kit formats for use with the QIAGEN BioRobot™ 96OO are also available. (See article on page 17)
¶ QIAquick Multiwell PCR Purification Kits for high-throughput purification are also available.