QIAGEN Supplementary Protocol:

Spin procedure for purifying 2 x 96 PCR samples using the Plate Rotor 2 x 96, a special centrifuge, and the QIAquick® 96 PCR Purification Kit

This protocol is designed for the simultaneous purification of multiple samples of single-stranded DNA or double-stranded PCR products of 100 bp to 10 kb from primers, nucleotides, polymerases, and salts using the QIAquick® 96 PCR Purification Kit.

Please be sure to read the *QIAquick Multiwell PCR Purification Handbook* and the detailed QIAquick 96 PCR Purification Kit Protocol carefully before beginning the procedure.

Equipment to be supplied by user

See ordering information on the last page.

- Centrifuge 4-15C or 4K15C*
- Plate Rotor 2 x 96
- Square-Well Blocks

Important notes before starting

- Centrifugation of QIAquick 96 Plates is performed at 6000 rpm (5788 x g). The speed limit of the Centrifuge 4-15C and Centrifuge 4K15C is programmed so that the given g-force will not be exceeded. All centrifugation steps are performed at room temperature.
- It is convenient to perform 2 x 96 preparations at one time since the rotor must always be balanced.
- Add ethanol (96–100%) to concentrated Buffer PE before use (see bottle label for volume).
- A reservoir or multichannel pipet facilitates liquid handling at many steps of the QIAquick 96 PCR Purification Kit procedure.

** The freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15, and 6K15 from Sigma Laborzentrifugen GmbH can also be used.*

Procedure

1. **Place QIAquick 96 Plate on top of a Square-Well Block. Mark the QIAquick 96 Plate for later identification.**

2. **Add 3 volumes of Buffer PM to 1 volume of PCR sample and mix. Pipet the samples into the wells of the QIAquick 96 Plate.**

   For example, add 300 µl of Buffer PM to 100 µl PCR sample (excluding oil volume). It is not necessary to remove mineral oil or kerosene.

3. **Load each Square-Well Block and QIAquick 96 Plate onto the carrier then place in the rotor bucket. Centrifuge at 6000 rpm for 4 min.**

4. **Empty the Square-Well Block. Add 900 µl of Buffer PE to each well. Centrifuge at 6000 rpm for 4 min.**

5. **Repeat step 4.**

6. **Place QIAquick 96 Plate on top of a collection microtube rack containing 1.2 ml collection microtubes. Incubate for 10 min at 70°C in an incubator or oven to dry the membrane.**

   IMPORTANT: This step removes residual Buffer PE from the membrane. Residual ethanol, from Buffer PE, may inhibit subsequent enzymatic reactions, such as sequencing.

7. **To elute, add 80 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each well of the QIAquick 96 Plate, allow to stand for 1 min, and centrifuge at 6000 rpm for 4 min.**

   Please note that the average volume of eluate is 60 µl using 80 µl of elution buffer.

   Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and pH 8.5. When using water to elute, make sure that the pH value is within this range, and store DNA at –20°C as DNA may degrade in the absence of a buffering agent. The purified DNA can also be eluted in TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0), but the EDTA may inhibit subsequent enzymatic reactions.

Troubleshooting guide

	Comments and suggestions
Low or no recovery	
Buffer PE did not contain ethanol	Ethanol must be added to Buffer PE (concentrate) before use. Repeat procedure with correctly prepared Buffer PE.
Inappropriate elution buffer	DNA will only be eluted efficiently in the presence of low salt and pH ≥7.0. Use the provided Buffer EB (10 mM Tris·Cl, pH 8.5) or water for elution. If water is used, make sure that the pH is between 7.0 and 8.5. RNase-free water is provided with QIAquick 96 PCR Purification Kits, giving the option of salt-free elution conditions.
Elution buffer not covering QIAquick membrane	Dispense/pipet elution buffer onto the center of each well of the QIAquick 96 Plate to ensure the membrane is completely covered.
DNA does not perform well in downstream applications	
Eluate contains residual ethanol	Take care to follow exactly step 6 in the protocol.
Eluate contains residual salt	Ensure that two wash steps (steps 4 and 5) are carried out prior to elution to remove salt completely.
Eluate contains primer-dimers	Primer-dimers formed are longer than 20 bp, and are not completely removed. After the binding step (step 3), wash the QIAquick wells with 750 µl of a 35% guanidine hydrochloride aqueous solution (35 g in 100 ml). Centrifuge and proceed with the Buffer PE wash step (step 4).

Ordering Information

Product	Contents	Cat. No.
QIAquick 96 PCR Purification Kit		
QIAquick 96 PCR Purification Kit (4)*†	For purification of 4 x 96 PCR samples: 4 QIAquick 96 Plates, Buffers, Collection Microtubes (1.2 ml), Caps	28181
Related products		
QIAquick 8 PCR Purification Kit (10)‡	For purification of 10 x 8 PCR samples: 10 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2 ml), Caps	28142
QIAquick 8 PCR Purification Kit (50)‡	For purification of 50 x 8 PCR samples: 50 QIAquick 8 Strips, Buffers, Collection Microtubes (1.2 ml), Caps	28144
Accessories		
Germany§ Centrifuge 4-15C (220 V)	Universal laboratory centrifuge with brushless motor (220 V, 50 Hz)	81020
USA§ Centrifuge 4-15C (120 V)	Universal laboratory centrifuge with brushless motor (120 V, 60 Hz)	81010
Germany§ Centrifuge 4K15C (220 V)	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 50 Hz)	81220
USA§ Centrifuge 4K15C (220 V)	Universal refrigerated laboratory centrifuge with brushless motor (220 V, 60 Hz)	81210
Plate Rotor 2 x 96¶	Rotor for 2 QIAGEN 96-well plates, for use with QIAGEN Centrifuges	81031
Square-Well Blocks (24)	96-well blocks with 2.2 ml wells, 24 per case	19573

* *Requires use of either QIAvac 96 or the Plate Rotor 2 x 96 and special centrifuge.*

† *Larger kit sizes available; please inquire.*

‡ *Requires use of QIAvac 6S. In other countries please contact your local subsidiary or distributor.*

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¶ *The Plate Rotor 2 x 96 is available exclusively from QIAGEN and its distributors. Under the current liability and warranty conditions, the rotor may only be used in Centrifuges 4-15C and 4K15C from QIAGEN, and freely programmable models of centrifuges 4-15, 4K15, 6-10, 6K10, 6-15 and 6K15 from Sigma Laborzentrifugen GmbH.*

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.
Selected handbooks can be downloaded from **www.qiagen.com/literature/handbooks/default.asp**.
Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from **www.qiagen.com/ts/msds.asp**.