QIAquick PCR Purification Kit Protocol

Fragments ranging from 100 bp to 10 kb are purified from primers, nucleotides, polymerases, and salts using QIAquick spin columns in a microcentrifuge.

Notes: • Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).

1) Add 5 volumes of Buffer PB*** to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene.

   For example, add 500 µl of Buffer PB to 100 µl PCR sample (not including oil).
2) Place a QIAquick spin column in a provided 2 ml collection tube.
3) To bind DNA, apply the sample to the QIAquick column and centrifuge for 30–60 seconds.
4) Discard flow-through. Place the QIAquick column back into the same tube.
5) To wash, add 750 µl Buffer PE to the QIAquick column and centrifuge for 30–60 seconds.
6) Discard flow-through and place the QIAquick column back in the same tube.
7) Centrifuge the column for an additional 2 minutes.

   IMPORTANT: *Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.*
8) Place QIAquick column in a clean 1.5 ml microcentrifuge tube.
9) To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or H_2O to the center of the QIAquick membrane and centrifuge the column for 1 minute. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 5-10 minutes, and then centrifuge.

   EB Buffer can be heated to 65°C before use to increase recovery

   IMPORTANT: *Ensure that the elution buffer is dispensed directly onto the QIAquick membrane for complete elution of bound DNA. The average eluate volume is 48 µl from 50 µl elution buffer volume, and 28 µl from 30 µl elution buffer. Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5.*

*** Using the PQ buffer from QiaQuick gel extraction kit instead of PB buffer gives better yield of recovery