Qiagen QIAquick Gel Extraction Kit (28704 and 28706), centrifuge processing

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Abstract

The Qiagen QIAquick Gel Extraction kit (catalog #28704 and 28706) are for extraction of DNA fragments (70 bp – 10 kb) from standard or low-melt agarose gels in TAE buffer (Tris·acetate/EDTA) or TBE buffer (Tris·borate/ EDTA) and DNA cleanup from enzymatic reactions. The kit is suitable for purification of up to 10µg of DNA (70bp to 10kb).

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Before start

Add ethanol (96–100%) to Buffer PE before use (see bottle label for volume).
All centrifugation steps are carried out at 17,900 x g (13,000 rpm) in a conventional tabletop microcentrifuge at room temperature (15–25°C).
Add 1:250 volume pH Indicator I to Buffer PB (i.e., add 120 µl pH Indicator I to 30 ml Buffer PB or add 600 µl pH Indicator I to 150 ml Buffer PB). The yellow color of Buffer PB with pH Indicator I indicates a pH of ≤ 7.5. Add pH Indicator I to entire buffer contents. Do not add pH Indicator I to buffer aliquots. If the purified PCR product is to be used in sensitive microarray applications, it may be beneficial to use Buffer PB without the addition of pH Indicator I.
Isopropanol (100%) and a heating block or water bath at 50°C are required

Protocol

Extraction

Step 1.
Excise DNA fragment from agarose gel with clean, sharp scalpel

Extraction

Step 2.
Weigh the gel slice in a colorless tube. Maximum amount of gel per column is 400mg (about 0.4cm^3)

Extraction

Step 3.
Add 3 volumes Buffer QG to 1 volume of gel (100mg gel 100µL). For >2% agarose gels, add 6 volumes Buffer QG

AMOUNT

3 µl Additional info:

Extraction

Step 4.

Incubate at 50°C for 10 minutes (or until gel slice has completely dissolved). [Optional]: Vortex every 2-3 min to help dissolve gel.

DURATION
00:10:00

Extraction

Step 5.
Check color of mixture to ensure it is yellow, similar to Buffer QG without dissolve agarose. If color is orange or violet, add 10µL of 3M sodium acetate, pH 5.0, and mix.

Extraction

Step 6.
For purifying <500bp or >4kb, add 1 volume isopropanol to increase yield and mix

AMOUNT
1 µl Additional info:

Binding

Step 7.
Place a QIAquick spin column in a provided 2 ml collection tube.

Binding

Step 8.
To bind DNA, apply the sample to the QIAquick column and centrifuge for 1 min then discard flow through. The maximum volume is 800µL. For larger volumes, load and spin again.

DURATION
00:01:00

Binding

Step 9.
Return QIAquick column to collection tube

Wash

Step 10.
If the DNA will subsequently be used for sequencing, *in vitro* transcription, or microinjection, add 500µL Buffer QG to the QIAquick column and centrifuge for one minute.

DURATION
00:01:00

Wash

Step 11.
Discard flow through and return column to collection tube

Wash

Step 12.
To wash, add 750 µl Buffer PE to the QIAquick column

REAGENTS
Buffer PE by Contributed by users

Wash

Step 13.
If DNA will be used for salt-sensitive applications (e.g. sequencing, blunt-ended ligation), let column stand for 2-5 minutes

DURATION
00:05:00

Wash

Step 14.
Centrifuge for 1 minute

DURATION
00:01:00

Wash

Step 15.
Discard flow-through and place the QIAquick column back in the same tube. Centrifuge the column for an additional 1 min.

IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation.

DURATION
00:01:00

Elution

Step 16.
Place QIAquick column in a clean 1.5 ml microcentrifuge tube.

Elution

Step 17.
To elute DNA, add 50 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water (pH 7.0–8.5) to the center of the QIAquick membrane and centrifuge the column for 1 min. Alternatively, for increased DNA concentration, add 30 µl elution buffer to the center of the QIAquick membrane, let the column stand for 1 min, and then centrifuge.

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. When using water, 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.

Analysis

Step 18.
If the purified DNA is to be analyzed on a gel, add 1 volume of Loading Dye to 5 volumes of purified DNA. Mix the solution by pipetting up and down before loading the gel.

Loading dye contains 3 marker dyes (bromophenol blue, xylene cyanol, and orange G) that facilitate estimation of DNA migration distance and optimization of agarose gel run time. Refer to Table 2 (page 15) to identify the dyes according to migration distance and agarose gel percentage and type.