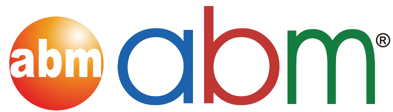

Column-Pure Gel and PCR Clean-Up Kit

Store at 18-25°C

Cat. No.	Description	Quantity
D516	Column-Pure Gel and PCR Clean-Up Kit	100 preps

Product Description

abm's Column-Pure Gel and PCR Clean-Up Kit is a quick and versatile system for the isolation and purification of DNA. This two-in-one kit can be used to purify high quality DNA following PCR amplification and agarose gel isolation. The silica spin column technology allows for rapid recovery of high quality DNA samples that are ready for downstream applications such as PCR, restriction digest, cloning and sequencing.

Kit Components

Component	Volume / Size
Binding Buffer	100 ml
Wash Buffer (concentrate)	40 ml
Elution Buffer	10 ml
Spin Columns and Collection Tubes	100

Caution

The Binding Buffer contains a chaotropic salt which can form highly reactive compounds when combined with bleach. Do not add bleach or acidic solutions directly to waste containing Binding Buffer.

Storage

Store all buffers at 18-25 °C (room temperature).

Protocol

Note: Before use, prepare 1X Wash Buffer by adding 160 ml of ethanol (95-100%) to the Wash Buffer bottle.

PCR Clean-Up

This procedure is suitable for PCR clean-up and concentrating/purifying DNA samples by removal of enzymes, salts and other impurities.

Perform all centrifugation steps at 12,000 rpm

1. Add Binding Buffer to PCR reaction in a 5:1 ratio. Vortex briefly.
   E.g. add 250 µl Binding Buffer to a 50 µl PCR reaction.
2. Load ≤700 µl of mixture to Spin Column. Centrifuge for 30 s. Discard flow-through.
   If the initial mixture volume is ≥700 µl, add the remaining volume to column and repeat spin.
3. Add 700 µl of 1X Wash Buffer to column and centrifuge for 30 s. Discard flow-through.
4. Repeat Step 3. Discard flow-through. Centrifuge for 1 min.

5. Transfer Spin Column to a clean 1.5 ml microcentrifuge tube.
6. Add 30-50 µl Elution Buffer to the center of column and incubate at room temperature for 1 min. Centrifuge for 1 min.
7. Store purified DNA at -20°C.

DNA Extraction from Agarose gels

This procedure is suitable for the isolation and purification of DNA following agarose gel electrophoresis.

Perform all centrifugation steps at 12,000 rpm

1. Use a clean scalpel to excise the DNA fragment from the agarose gel.
2. Weigh gel slice. Transfer to a clean 1.5 ml microcentrifuge tube.
3. Add Binding Buffer to gel slice in a 4:1 ratio.
   E.g. add 400 µl of Binding Buffer to 100 mg of gel.
   If the agarose concentration is >1.5%, use a 7:1 ratio of Binding Buffer.
4. Incubate mixture at 55-60°C for 3 min or until the gel slice is completely dissolved.
   To increase efficiency, cut the gel slice into smaller pieces (Step 1) and vortex periodically.
5. Load ≤700 µl of mixture to Spin Column and centrifuge for 30 s. Discard flow-through.
   If the initial mixture volume is ≥700 µl, add the remaining volume to column and repeat spin.
6. Add 700 µl of 1X Wash Buffer to column and centrifuge for 30 s. Discard flow-through.
7. Repeat Step 6. Discard flow-through. Centrifuge for 1 min.
8. Transfer Spin Column to a clean 1.5 ml microcentrifuge tube.
9. Add 30-50 µl of Elution Buffer to the center of column and incubate at room temperature for 1 min. Centrifuge for 1 min.
10. Store purified DNA at -20°C.

Note: It is important to add the Elution Buffer into the center membrane of the column. To increase yield, elution can be performed with 15 µl, provided the elution step is repeated by reloading the eluent into the same column and centrifuging for 1 min. Incubating the column with the Elution Buffer at higher temperatures (37-50°C) may slightly increase the yield especially for large (>10 kb) plasmids. Pre-warming the Elution Buffer at 55-60°C may slightly increase elution efficiency.

Tips on Gel Extraction
- *Run agarose gels in clean, fresh 1X TAE buffer*
- *Use lower voltages (50-75 V) and longer run times for improved DNA fragment separation*
- *Use a clean scalpel or blade to excise the DNA fragment*
- *Minimize or avoid UV exposure of the DNA fragment*
- *Minimize the amount of agarose excised with the DNA fragment*