Column-Pure Gel and PCR Clean-Up Kit

Cat. No. D516
Store all components at 18-25°C.

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.

Product Component	Quantity
Binding Buffer	100 ml
Wash Buffer	40 ml
Elution Buffer	10 ml
Spin Columns and Collection Tubes	100

Protocol
Before use, add 160 ml of ethanol (95-100%) to the Wash Buffer.
Perform all centrifugation steps at 12,000 rpm.

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

1. Add 5 volumes of Binding Buffer to 1 volume of PCR reaction and vortex briefly.
   •*E.g. Add 250 µl Binding Buffer to 50 µl PCR reaction.*
2. Apply ≤700 µl of mixture to Spin Column and centrifuge for 30 s. Discard flow-through.
   •*Repeat Step 2 until all remaining mixture has been applied to Spin Column.*
3. Add 700 µl of Wash Buffer (with added ethanol) to Spin Column and centrifuge for 30 s. Discard flow-through.
4. Repeat Step 3. Centrifuge Spin Column for 1 min to remove residual Wash Buffer.
5. Discard the Collection Tube and transfer the Spin Column into a clean 1.5 ml microcentrifuge tube.
6. Add 30-50 µl of Elution Buffer to the center of the Spin Column. Incubate for 1 min at room temperature and 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.

1. Excise the DNA fragment from the agarose gel using a clean scalpel and place into a 1.5 ml microcentrifuge tube.
2. Weigh the gel slice and add 4 volumes of Binding Buffer to 1 volume of gel slice.
   •*E.g. Add 400 µl Binding Buffer to 100 mg of gel.*
3. Incubate mixture at 55-60°C for 3 min or until the gel slice is completely dissolved.
4. Vortex briefly and apply ≤700 µl of mixture to Spin Column and centrifuge for 30 s. Discard flow-through.
   •*Repeat Step 4 until all remaining mixture has been applied to Spin Column.*
5. Add 700 µl of Wash Buffer (with added ethanol) to Spin Column and centrifuge for 30 s. Discard flow-through.
6. Repeat Step 5. Centrifuge Spin Column for 1 min to remove residual Wash Buffer.
7. Discard the Collection Tube and transfer the Spin Column into a clean 1.5 ml microcentrifuge tube.
8. Add 30-50 µl of Elution Buffer to the center of the Spin Column. Incubate for 1 min at room temperature and centrifuge for 1 min.
9. Store purified DNA at -20°C.

General Notes
- To increase yield: pre-warm the Elution Buffer to 55-60°C. Apply 15-20 µl pre-warmed Elution Buffer directly to the center of the Spin Column membrane, centrifuge for 1 min, then reapply the eluate to the same Spin Column and centrifuge for an additional 1 min.
- To increase purity: run agarose gel in a clean electrophoresis chamber with fresh 1X TAE Buffer, use a clean scalpel and minimize the amount of agarose excised with the DNA fragment.
- For cloning applications: it is important to avoid using UV exposure when excising the DNA fragment from the agarose gel in order to avoid damaging the DNA.

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