Geneflow Gel/PCR DNA Purification Kit

The Kit is designed for purification of DNA up to 10 Kb from agarose gels or reaction solutions (PCRs or restriction digests).

Components	K1-0030	K1-0040
Spin Columns	50	250
2-ml Collection Tubes	50	250
Binding Buffer	30 ml	3x50 ml
Wash Solution I, concentrate	8 ml	5x8 ml
Elution Buffer, 5 mM Tris-Cl, pH 8.0	5 ml	25 ml

Notes before use:

1. Add 50 ml of ethanol (96-100%) to 8 ml of Wash Solution I concentrate.
2. TE buffer is not recommended for elution.
3. Benchtop microcentrifuge (7,000-12,000 rpm) can be used for all following procedures.

Protocol

1. **For DNA extraction from the gel:** Excise the DNA fragment from the agarose gel and transfer to a clean microfuge tube. Weight the gel slice and add equal volume of Binding Buffer, e.g 100 µl of the Buffer per 100 mg of gel slice. Excess of the Binding Buffer is preferable. For concentration of agarose >1.5% add twice the volume of the Binding Buffer.
2. Incubate with occasional vortexing for 5-10 min in a water bath (50-65 °C) or until the gel is completely dissolved.
3. **For DNA purification from reaction mixes (PCR or digests):** Add equal volume of Binding Buffer, vortex or mix with pipetting. Incubate at RT for 1 min.
4. Add the above mixtures to the spin column and let to stand at RT for 2 min.
5. Centrifuge for 1 min and discard the flow-through in the tube.
6. Add 500 µl of Wash Solution I and spin for 15 seconds. Discard the flow-through solution.
7. Repeat step 6.
8. Centrifuge for 1 minute to remove residual Wash Solution I.
9. Transfer the column into 1.5 ml microfuge tube, cut the tube's cap with scissors if necessary. Add 30-50 µl of Elution Buffer on to the center part of membrane of the column and incubate for 1- 2 min at room temperature. Spin for 1 min.
10. Transfer the plasmid DNA into a clean tube and store at –20 °C.

Troubleshooting:

Low DNA yield	DNA fragments smaller than 100 bp and bigger than 10 kb may lead to lower recovery of DNA. Increase the incubation time with Elution Buffer. Preheat column and Elution Buffer at 50-60°C before use.
Gel is difficult to dissolve	Add more Binding Buffer and repeat solubilisation step 2.

For research use only. **March 2006**

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