QuickClean 5M Gel Extraction Kit

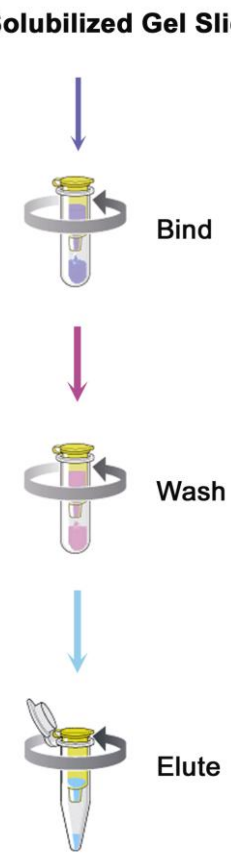
Technical Manual No. 0183 **Version 02052009**

I. DESCRIPTION

QuickClean 5M Gel Extraction Kit is a reagent kit for fast DNA extraction and purification from agarose gels (standard or low-melt). Up to 20 µg DNA (both double-stranded and single-stranded, 70 bp – 10 kb) can be recovered in five minutes from solubilized gel slices. The kit is compatible with agarose gels with either TBE or TAE as running buffers. The QuickClean Column efficiently absorbs DNA molecules of between 70 and 10,000 base pairs. Other impurities such as agarose, dyes, and salts are washed away.

Eluted in a small volume low-salt buffer with complete removal of contaminants and inhibitors, the purified DNA is ready for most downstream applications such as PCR, transformation, restriction, enzyme digestion, cloning, sequencing, *in vitro* translation, and transfection.

Overview of Kit Procedure



II. KIT CONTENTS

The QuickClean 5M Gel Extraction Kit contains sufficient reagents, mini-columns, and collection tubes for 250 purifications (using up to 200 mg of agarose for each purification):

L00199 Components	250 Preps
binding solution II	2 x 110 ml
wash solution	55 ml
elution buffer	25 ml
QuickClean columns	250
2 ml collection tubes	250
protocol	1

III. APPLICATIONS

The QuickClean 5M Gel Extraction Kit enables the fast extraction and purification of high-quality DNA from agarose gels (using either TBE or TAE as the running buffer). The extracted DNA is immediately ready for further downstream applications:

- PCR and cloning
- Restriction enzyme digestion
- Transformation
- Sequencing
- *In vitro* translation

IV. KEY FEATURES

- Easy to perform: QuickClean's simple and rapid procedure purifies DNA in five minutes.
- High capacity: Each column has a capacity of 20 µg DNA.
- High purity: The kit completely removes contaminants and inhibitors.
- Reproducible yields: Recovery is typically between 70% and 90%, reproducible every time.

V. STORAGE

This kit should be stored dry at room temperature. So stored, the kit is stable for 12 months.

VI. PURIFICATION PROTOCOL

The following steps may be performed in advance:

1. Add 220 ml of 96-100% ethanol to 55 ml of the wash solution and mix well.
2. Some precipitate may form in the binding solution after long periods of storage. Dissolve the precipitate by mixing gently. Otherwise, warm the container to 37°C for a few minutes.

Procedure:

1. Using a clean, sharp razorblade or scalpel, excise the DNA band from the agarose gel. Remove excess agarose.
2. Place the gel slice in a colorless, pre-weighed tube and weigh the gel slice. Add three volumes of binding solution II to one volume of gel slice (100 mg ≈ 100 µl). For gels containing more than 2% agarose, add six volumes of binding solution II.
3. Incubate at 50°C for ten minutes with occasional vortexing or until the gel slice has completely dissolved. Usually, the color of the mixture will be yellow. If the color of the mixture is violet, add 5 to 10 µl of 3M sodium acetate (pH 5.0) and mix. Then it will turn to yellow.
4. Add one volume of isopropanol (with respect to the original gel volume) and mix. Transfer the mixture (if less than 600 µl) to QuickClean column and centrifuge at 12,000 rpm for 30 seconds. For mixture volumes of more than 600 µl, load and centrifuge again using the same column.
5. Discard the flow-through from the tube. Add 500 µl of wash solution to the column and centrifuge at 12,000 rpm for one minute. If necessary, repeat wash procedure once.
6. Remove and discard the flow-through. Centrifuge at 12,000 rpm for additional 60 seconds to remove residual wash solution.
7. Transfer the column to a clean 1.5 ml microcentrifuge tube. Add 30 to 50 µl elution buffer to the center of the column membrane and incubate at room temperature for one minute. Centrifuge at 12,000 rpm for one minute to elute and collect DNA.

Note: If residual ethanol is present in the elution solution, it may complicate the loading into gels for analysis. The loading solution may rise or "blow up," instead of sinking smoothly into the wells of gel. The addition of more loading buffer can prevent this phenomenon. For example, use 5X loading buffer as 3X loading buffer or double the volume of loading buffer.

VII. EXAMPLES

The QuickClean 5M Gel Extraction Kit is here compared with a commercially available kit (Competitor A) for DNA extraction and purification from agarose gel following the protocols provided by the manufacturers.

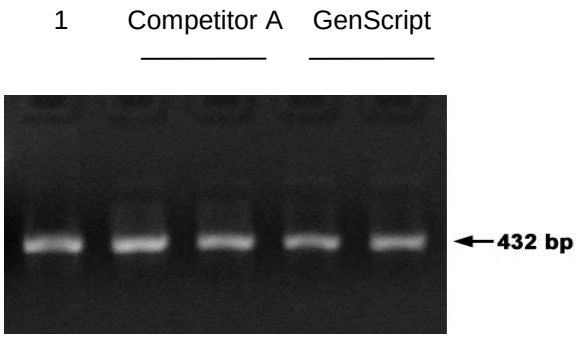


Figure 1. The QuickClean 5M Gel Extraction Kit and a competing product extracted and purified DNA from agarose each following the manufacture's protocols.
Two DNA purifications were performed using each kit.
The same amount of DNA was loaded in four wells of the agarose gel used in this test. Lane 1 shows an unpurified sample.

VIII. TroubleshootinG

Use the table below to solve and avoid common problems.

Problem	Probable Cause	Solution
Low DNA recovery	Not enough binding solution was used or isopropanol was not added.	Add three volumes of binding solution II to the gel slice. For gels containing more than 2% agarose, add six volumes of binding solution II. After the gel has completely dissolved, add one volume of isopropanol and mix.
	Gel slice not completely dissolved.	During incubation at 50 ℃, mix by vortexing or inverting the tubes every two minutes. Make sure that the gel slice completely dissolved.
	The wash solution did not contain ethanol.	Before use, add 220 ml of 96-100% of ethanol to 55 ml of wash solution and mix well. Put a check mark in the box on the cap of the wash solution bottle.
	The solubilized gel solution was highly basic.	If the solubilized gel solution has a violet color, add 5 to 10 µl of 3M sodium acetate (pH 5.0) and mix. The color of the mixture should be yellow for efficient binding.
	Another elution solution was used.	Elution buffer is 2.0 mM Tris-HCl pH 8.5. TE buffer (pH 8.0) or water can also be used, but the yield may be slightly lower.
	The gel slice is too large.	Repeated loadings of the solubilized gel solution to the same column will reduce the total yield of DNA. Trim the gel slice to remove extra agarose.

IX. ORDERING INFORMATION

QuickClean 5M Gel Extraction Kit Catalog Number: L00199

For Research Use Only.

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