

MEGAquick-spin™

PCR & Agarose Gel DNA Extraction System

Cat. No. 17281 50 Columns
Cat. No. 17282 250 Columns

DESCRIPTION

The MEGAquick-spin PCR and Agarose Gel DNA Extraction system is designed to extract and purify DNA fragments of 87 bp to 20 kb from normal or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBE), or to purify PCR products directly from a PCR amplification. Up to 95% recovery is achieved depending upon the fragment size. PCR products are commonly purified to remove excess nucleotides and primers.

This spin column-based system, which can bind up to 40 ug of DNA, allows recovery of isolated DNA fragments or PCR products in as little as 20 minutes, depending on the number of samples processed and the protocol used. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, restriction enzyme digestion or routinely performed DNA manipulation.

STORAGE

Store at RT.

The vinyl bag contained spin column should be sealed tightly.

KIT CONTENTS

- BNL Buffer 40 ml (170 ml)
: Agarose gel lysis, DNA binding buffer.
- Washing Buffer 10 ml (40 ml x 2ea)
: Add 40 ml (160 ml/each bottle) of absolute EtOH to Washing Buffer prior to beginning the procedure. Mark the bottle label to record that this addition was made. Tightly close the bottle cap after each use to prevent evaporation.
- Elution Buffer 20 ml
- Columns (Blue) 50 Columns (250 Columns)
: Columns containing silica-membrane, The maximum volume of the column reservoir is 800 µl.
- Collection tubes 50 Tubes (250 Tubes)
: Polypropylene tube for 2 ml.

PROTOCOL

A. Dissolving the Gel Slice (Agarose Gel DNA Purification)

1. Load and run the gel using an established protocol. DNA can be extracted from standard or low-melt agarose gels run with either TAE or TBE buffer.
2. Weigh a 1.5 ml microtube for each DNA fragment to be isolated and record the weight.
3. Visualize and photograph the DNA using a long-wavelength UV lamp and an intercalating dye such as ethidium bromide. To reduce nicking, irradiate the gel for the absolute minimum time possible. Excise the DNA fragment of interest in a minimal volume of agarose using a clean scalpel or razor blade. Transfer the gel slice to the weighed microcentrifuge tube and record the weight. Subtract the weight of the empty tube from the total weight to obtain the weight of the gel slice.
Note: The gel slice may be stored at 4 °C or at -20 °C for up to one week in a tightly closed tube under nuclease-free conditions before purification.
4. Add BNL Buffer at a ratio of 300 ul of solution per 100 mg of agarose gel slice. (3 volumes of BNL Buffer to 1 volume of gel)
Note: For > 2% agarose Gel, add 6 volumes of BNL Buffer. The maximal amount of gel slice per MEGAquick-spin column is 400 mg ; for gel slice > 400 mg use more than one MEGAquick-spin column.
5. Vortex the mixture and incubate at 55 °C for 10 minutes or until the gel slice is completely dissolved.
Note: Vortex the tube every few minutes to increase the rate of agarose gel melting. Centrifuge the tube briefly at room temperature to ensure the contents are at the bottom of the tube. Once the agarose gel is melted, the gel will not resolidify at room temperature.

6. (Optional) Add 1 gel volume of isopropanol to the step 5 dissolved gel solution and mix well by pipetting several times. Do not centrifuge after mixing well.

Note: For < 200 bp of DNA fragment, add 1 volume of isopropanol to 1 volume of gel, and mix well. If the agarose gel slice is 100 mg, add 100 ul of isopropanol. When adding the isopropanol and mixing well by pipetting, small white pellet and clump should be form. But never mind, go to the following step. This step increases the yield of DNA fragment. For DNA fragment > 200 bp, addition of isopropanol has no effect on yield.

7. To purify the DNA using a microcentrifuge, proceed to [Section C](#).

B. Processing PCR Reactions (PCR Product Purification)

1. Amplify target of choice using standard amplification conditions.
2. Add an 5 volume of BNL Buffer to the PCR reaction product, and mix well by vortexing. If the PCR product is 20 ul, add 100 ul of BNL buffer to the PCR tube directly.
Note: Centrifuge the tube briefly at room temperature to ensure the contents are at the bottom of the tube.
3. (Optional) For < 200 bp, Add 1.5 volume of isopropanol to the sample and mix well by pipetting several times. Do not centrifuge after mixing well.
Note: For < 200 bp, Add 1.5 volume of isopropanol, and mix well. If the PCR product is 20 ul, add 100 ul of BNL Buffer and 150 ul of isopropanol. This step increases the yield of DNA fragment.
3. To purify the DNA using a microcentrifuge, proceed to [Section C](#).

C. DNA Purification by Centrifugation

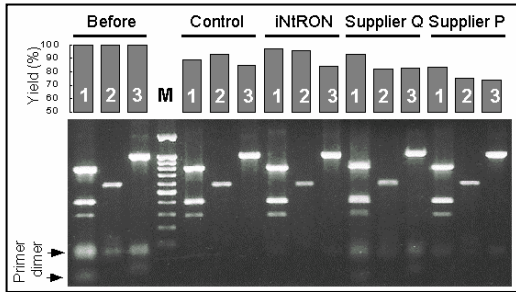
1. Place one spin column (blue color) in a Collection Tube for each dissolved gel slice or PCR reaction product.
2. Transfer the dissolved gel mixture or prepared PCR product to the spin column assembly.
3. Load the sample to the spin column and centrifuge at 13,000rpm for 1min. Discard the flow-through after centrifuging and place the spin column back in the same 2 ml collection tube.
Note: The maximum volume of the column reservoirs is 800 ul. For larger volume, sample reload and spin again.
4. Add 700 ul of Washing Buffer to column and centrifuge at 13,000rpm for 1min. Discard the flow-through after centrifuging and place the spin column back in the same 2 ml collection tube.
Note: If the DNA will be used for salt sensitive applications, such as blunt-end ligation and direct sequencing, repeat the step 4 using 500 ul of Washing buffer.
5. Centrifuge for 1 min at 13,000rpm to dry the spin membrane.
Note: It is important to dry the spin membrane since residual ethanol may interfere with other reactions.
6. Place the spin column to a clean 1.5 ml microcentrifuge tube (not provided). Apply 30-100 ul of the Elution Buffer directly to the center of the column without touching the membrane with the pipette tip. Incubate at room temperature for 1 minute. Centrifuge for 1 minute at 13,000rpm.
7. Discard the spin column and store the microcentrifuge tube containing the eluted DNA at -20 °C .
Note: It is suggested to use at least 20 ul of the Elution Buffer to obtain best result

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

Complete primer removal after PCR

MEGAquick-spin™ PCR & Agarose Gel DNA Extraction System shows not only reliable DNA recovery but complete primer removal efficacy



▲ Figure 1. Analysis of PCR product.

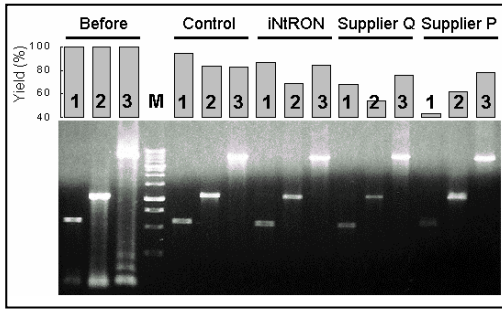
The bar-graph shows the recovery of DNA fragment. The values of yield was estimated with TINA 2.0 software.

Before. Before purification; **Control.** PCRquick-spin™ PCR product Purification Kit (iNtRON); **iNtRON.** MEGAquick-spin™ PCR & Agarose Gel DNA Extraction System; **Supplier Q and P.** Q and P company products.

Lane 1. Multiplex PCR product Lane 2. 570 bp fragment
Lane 3. 1.0 kb fragment Lane M. 100 bp Ladder Molecular Weight DNA Marker

Adequate DNA recovery

MEGAquick-spin™ PCR & Agarose Gel DNA Extraction System assures of the suitable recovery of DNA fragment from agarose gel



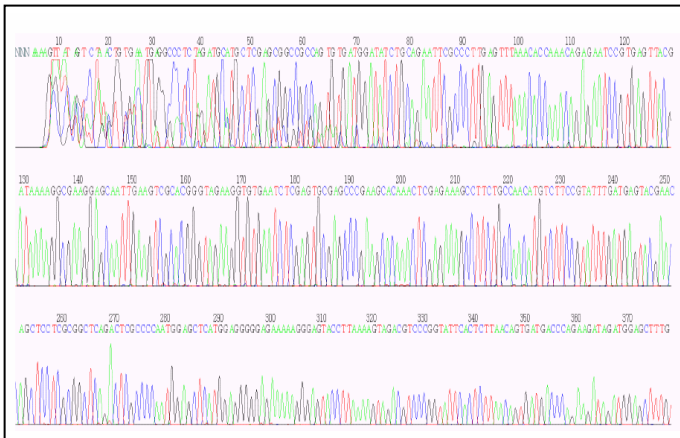
▲ Figure 2. Size exclusion by agarose gel extraction.

The bar-graph shows the recovery of DNA fragment. The values of yield was estimated with TINA 2.0 software.

Before. Before purification; **Control.** MEGA-spin™ Agarose Gel DNA Extraction Kit (iNtRON); **iNtRON.** MEGAquick-spin™ PCR & Agarose Gel DNA Extraction System; **Supplier Q and P.** Q and P company products.

Lane 1. 570 bp fragment Lane 2. 1.3kb fragment
Lane 3. 4.5 kb fragment Lane M. 1 kb Ladder Molecular Weight DNA Marker

The purity is enough to perform following application

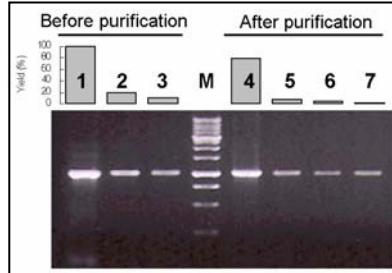


▲ Figure 3. The automated DNA sequence analysis.

The purified DNA fragment (4.5 kb size) was analyzed by automated DNA sequencing (ABI PRISM #377). Up to 600 bases of considerable DNA sequences were obtained, respectively

Maximum Yield

The first fraction (lane 4) showed app. 80% of recovery, but the recovery was increased up to 95% by repeated elution.



◀ Figure 4. The Additional elution for recovery enhancing.

After 1st elution, another elution was repeated by adding elution buffer, newly. Lane 1. 100% of DNA; Lane 2. 20% of DNA; Lane 3. 10% of DNA; Lane 4. 1st eluate; Lane 5. 2nd eluate; Lane 6. 3rd eluate; Lane 7. 4th eluate; Lane M. 1 kb Ladder Molecular Weight DNA marker

TROUBLESHOOTING GUIDE

| Problem | Possible Cause | Recommendation |
|---|--|---|
| Low or no DNA yield | Washing buffer did not contain ethanol | - Ethanol must be added to Washing Buffer before use. |
| | Inappropriate elution buffer | - DNA will only be eluted in low salt buffer or water. |
| | Incorrect volume of BNL buffer | - Verify that a correct volume of BNL Buffer was added to the Gel slice or PCR product (Refer to section A-4 or B-5) |
| | Gel slice incompletely solubilized | - After addition of BNL buffer to the slice, mix by vortexing the tube every 2 minutes during the 55 °C incubation. |
| DNA does not perform well, e.g., in enzyme reaction, ligation, Sequencing reactions | Cloudy and gelatinous appearance of sample mixture after addition of isopropanol | - This may be due to salt contamination, and will be disappear upon mixing the sample. Alternatively, the gel slice may not be completely solubilized. (the concentration of gel may be above 2%). In this case, apply the 6 volume of BNL buffer to gel slice, and melt the gel completely |
| | Salt concentration in eluate too high | - Modify the wash step by incubating the column for 5 min at RT after adding 700 ul of Washing Buffer, the centrifuge. |
| | Eluate contaminated with agarose | - The gel slice is incompletely solubilized or over-weighed. Repeat procedure from step. 1. |
| Eluate contains residual ethanol | Eluate contains residual ethanol | - Ensure that the wash flow-through is drain from the collection tube and that the MEGAquick-spin column is then centrifuged at 13,000rpm for 1min. |
| | Eluate contain primer-dimers | - Primer dimers formed are longer than 50bp, and are not completely removed. After binding step, wash the the MEGAquick-spin column with 750 ul of a 35% Guanidine hydrochloride aqueous solution. Follow with the washing, and elution step as in the protocol. |

RELATED PRODUCTS

| Product Name | Cat.No. |
|--|---------|
| Muta-Bulk™ Insert & Deletion Mutagenesis Kit (PreMix Type) | 15072 |
| Muta-Direct™ Site-Directed Mutagenesis Kit | 15071 |
| PCRquick-spin™ PCR Product Purification Kit | 17202 |
| MEGA-spin™ Agarose Gel DNA Extraction Kit | 17183 |
| DNA-spin™ Plasmid DNA Purification Kit | 17093 |
| 100 bp Ladder Molecular Weight DNA Marker | 24012 |
| 1 kb Ladder Molecular Weight DNA Marker | 24022 |