Quantum Prep™ Gel Slice Kit

Instruction Manual

Catalog Number 732-6160

For Technical Service
Call Your Local Bio-Rad Office or
in the U.S. Call **1-800-4BIORAD**
(1-800-424-6723)

BIO-RAD

Table of Contents

Section 1
Safety

1.1 Health Hazard Information

For safety information, please refer to the appropriate material safety data sheet (MSDS).

1.2 Special Handling Information

Eye Protection

Safety glasses with splash guards. Maintain eye wash fountain and quick-drench facilities in work area.

Gloves

Chemical resistant gloves such as neoprene.

Protective Measures During Maintenance of Contaminated Equipment

Proper protective clothing, eye protection and respiratory equipment should be worn if working with large amounts of material.

Section 2
Introduction

2.1 Overview

The Quantum Prep Gel Slice kit contains reagents specifically designed for the extraction and purification of DNA electrophoresed on TAE or TBE agarose gels. The Quantum Prep gel slice matrix provides a quick and efficient way to recover double-stranded DNA fragments ranging in size from 50 bp to 23 kbp in less than 20 minutes.

2.2 Kit Components

The Quantum Prep Gel Slice kit contains sufficient reagents to perform 100 agarose gel extractions of up to 2 micrograms each. The following items are shipped with this kit:

Quantum Prep Gel Slice Matrix in 5.7 M guanidine thiocyanate, 25 mM acetate (pH 5.2)	100 ml
Quantum Prep Midi-Column spin filter columns without caps	100
2 ml microcentrifuge tubes with caps	100

Quantum Prep Wash Buffer 40 mM Tris, 4 mM EDTA, and 800 mM NaCl, pH 7.5 (before dilution with 95-100% ethanol). Final concentrations after the addition of 1 x volume of ethanol: 20 mM Tris, 2 mM EDTA, 400 mM NaCl (pH 7.5) and 50% ethanol (v/v).	63 ml
Quantum Prep Elution Buffer 10 mM Tris and 1 mM EDTA, pH 8.5	6 ml

2.3 Additional Items Required but Not Provided

95–100% ethanol	Used to reconstitute Quantum Prep wash buffer.
Centrifuge tubes	Most 1.5 ml polypropylene microcentrifuge tubes are sufficient (catalog number 223-9480).
Centrifuge	Most standard benchtop microcentrifuges are sufficient
Vortex mixer	For matrix suspension purposes.
Water bath or heating block	55–70 °C for melting of agarose gel slice and incubation of matrix/DNA complex mixture.
Isopropanol	Used to wash Quantum Prep Gel Slice matrix/DNA.

2.4 Storage and Stability

All components are guaranteed for 12 months from the date of purchase, when stored and used as described in this manual.

Quantum Prep Gel Slice Matrix

Before using the Quantum Prep Gel Slice matrix, resuspend the matrix thoroughly by vigorous vortexing or inversion. This will ensure a uniform concentration of

matrix and will remove any aggregated matrix particles. Inverting the bottle after vortexing will ensure that all matrix has been resuspended.

Warning: The matrix is in 5.7 M guanidine thiocyanate, which is a strong oxidizer, and is harmful. Avoid contact and inhalation. Store the matrix away from reducing agents and strong acids. Wear gloves and safety glasses. See material safety data sheets (MSDS) for proper disposal of this material.

Quantum Prep Wash Buffer

Dilute the Quantum Prep wash buffer 1:1 with 95–100% ethanol before use. The pH of this wash buffer will be approximately 7.45 ± 0.15 at room temperature. Store the wash buffer in the freezer (-20 °C).

Quantum Prep Gel Slice Elution Buffer

No preparation is required. Store the elution buffer at room temperature. For long term storage, place at 4 °C.

Section 3
Protocol

3.1 Recommendations for Best Results

- Do not overload the Quantum Prep Gel Slice matrix. If the amount of DNA in the band of interest is greater than 2,000 ng (2.0 µg), split the sample into two tubes after excising and trimming, then proceed with the protocol.
- Always be sure to vortex the Quantum Prep Gel Slice matrix immediately before pipetting so that the correct amount of matrix is added. Pipette it slowly so that the correct volume is attained.
- Isopropanol is added at step (6) to facilitate hydrophobic binding interactions between sample DNA and the matrix. Final yield of purified sample can be increased in some cases by up to 15% by repeating step (6).
- To ensure that all traces of the ethanol have been removed from the sample, a final 1 minute centrifugation step is added after the two 30 second spins for removing the wash buffer. This drying spin step eliminates the possibility that residual wash buffer might be touching the bottom of the spin column, thus inhibiting complete drying of the pellet.

Such drying does not hinder the release of DNA from Quantum Prep Gel Slice matrix; However, it is not recommended to let the pellet dry for more than 30 minutes.

- The total sample volume should not exceed 1200 µl (2 x 600 µl) after the wash buffer has been applied at step (7). If, for some reason, you have more than 1200 µl, apply the remainder of the sample just before the final 1 minute drying spin. The maximum recommended capacity of the spin basket is 600 µl (to prevent spills).
- Add Quantum Prep Gel Slice elution buffer to the center of the matrix to ensure that the elution buffer covers the entire pellet. This is particularly important when using a small volume of elution buffer (30 µl) in order to maximize concentration of the sample.

3.2 Protocol—Isolation of DNA from TAE or TBE Agarose Gel Slices

1. Dilute the wash buffer 1:1 with 95–100% ethanol before use.
2. Using a clean razor blade, carefully excise the desired DNA band from an ethidium bromide stained agarose gel and place it into a clean 1.5 ml microcentrifuge tube. Trim excess agarose from all six sides of the DNA band to maximize recovery and purity. If

starting material contains more that 2,000 ng of DNA, split the sample into two tubes (see section 2.1)

3. Ensure that the Quantum Prep Gel Slice matrix is well suspended then add 900 µl of the matrix to the tube containing the gel slice. Vortex briefly.
4. Incubate at 55–70 °C until the gel slice has completely dissolved, usually 5–10 minutes. The total amount of time to dissolve will depend on the type and percentage of agarose used. Occasional inversions, flicking or vortexing of the tube during the melting step will facilitate binding of DNA to the matrix.
5. Pellet the DNA-containing Quantum Prep Gel Slice matrix by centrifuging for 1 minute in a microcentrifuge at 1,000 x g (~3,500 rpm). Remove the supernatant with a pipette tip attached to a vacuum aspirator, or with a pipette or disposable transfer pipette (catalog number 223-9527).
6. Add 1 ml of isopropanol to the pellet and vortex for 5–10 seconds. Pellet the sample by centrifuging for 1 minute in a microcentrifuge at 1,000 x g (~3,500 rpm). Remove the supernatant (isopropanol).

Optional: Repeat step (6) to achieve maximum yield (see section 2.1)

7. Add 1 ml of cold Quantum Prep wash buffer to the pellet from step (6) and vortex for 10–15 seconds.

8. Place the Quantum Prep Midi spin column into the 2 ml microcentrifuge tube (both provided) and apply 600 µl (maximum recommended) of the sample from step (7) to it. Centrifuge 30 seconds at 10,000 x g (13,000 rpm). Discard the flow-through. Repeat this step, then apply the remainder of the sample.
9. To insure that all traces of the ethanol have been removed, discard the flow-through from the second spin in step (8) and centrifuge 1 minute at 10,000 x g (13,000 rpm).
10. Discard the 2 ml tube and place the Quantum Prep Midi spin column in a clean 1.5 ml microcentrifuge tube.
11. Pre warm the Gel Slice elution buffer to 55 °C.
12. To elute the bound DNA, add 50 µl of the warm Gel Slice elution buffer or distilled water (or buffer of choice) to the center of the pellet. Let the column stand for 2 minutes, then centrifuge at approximately 10,000 x g (13,000 rpm) for 4 minutes.

Section 4
References

1. U.S. Patent 5,075,430 issued to Bio-Rad Laboratories.
2. Birnboim, H. C. and Doly, J., *Nuc. Acids Res.*, **3**, 1513, (1979).
3. Vogelstein, B. and Gillespie, D., *Proc. Natl. Acad. Sci. USA*, **76**, 615, (1979).
4. Willis, E. H., Mardis, E. R., Jones, W. L. and Little, M.C., *BioTechniques*, **9**, 1, (1990).
5. Jaggar, R. T. and MacDonald, C., *Nature*, (UK Product Review), **24**, (1992).
6. Carter, M. J. and Milton, I. D., *Nuc. Acids Res.*, **21**, 1044 (1993).

Section 5
Product Information

Catalog Number	Product Description
732-6160	**Quantum Prep Gel Slice Kit,** 1
732-6161	**Quantum Gel Slice Binding Matrix,** 1
732-6116	**Quantum Prep Midi Spin Columns,** 20 with caps
732-6024	**Quantum Prep Wash Buffer,** 250 ml
732-6165	**Quantum Prep Freeze & Squeeze DNA Gel Extraction Spin Column,** 25 columns
732-6166	**Quantum Prep Freeze & Squeeze DNA Gel Extraction Spin Column,** 100 columns
732-6300	**Quantum Prep PCR Kleen Spin Column,** 25 columns
732-6301	**Quantum Prep PCR Kleen Spin Column,** 100 columns

Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547
4006140 Rev B