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Zymoclean Gel DNA Recovery--CHEM 584

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ABSTRACT

Product Description

The Zymoclean™ Gel DNA Recovery Kit provides a hassle-free method for high yield recovery of pure DNA from agarose gels. Simply add the specially formulated Agarose Dissolving Buffer (ADB) to the gel slice containing your DNA sample, let dissolve, and then transfer to the supplied Zymo-Spin™ Column. There is no need for organic denaturants or chloroform. Instead, the product utilizes Fast-Spin column technology to yield high-quality DNA in just 15 minutes. DNA
purified using the Zymoclean™ Gel DNA Recovery Kit is perfectly suited for use in DNA ligation reactions, sequencing, DNA labeling reactions, PCR, etc.

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GUIDELINES

Specifications

- DNA Purity – High-quality, purified DNA is especially well suited for sequencing and ligation reactions.
- DNA Size Limits – From ~50 bp to 23 kb.

• DNA Recovery – Typically, up to 5 µg total DNA per column can be eluted into as little as 6 µl of low salt DNA Elution Buffer or water. For DNA 50 bp to 10 kb, the recovery is 70-90%. For DNA 11 kb to 23 kb, the recovery is 50-70%.

• Sample Sources – DNA in excised agarose gel slices.

• Product Detergent Tolerance – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.

Troubleshooting

Low Recovery

• Ensure Agarose is Fully Dissolved. There may be small globules of undissolved agarose in the sample that can interfere with DNA recovery by clogging the column and leeching salts into the eluate.

• Gel Dissolved at Temperatures Above 60 °C If dissolved at a higher temperature, DNA may be denatured affecting recovery. For optimal results, dissolve the gel slice between 37-55 °C.

• Improperly Stored DNA Wash Buffer. Cap the bottle tightly to prevent evaporation over time.

• Addition of DNA Elution Buffer. Add elution buffer directly to the column matrix, not to the walls of the column. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10kb.

• Incomplete Elution. (1) DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) to the column and incubate for several minutes prior to elution. (2) Sequential elutions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

Low A260/A230 ratio

• Column tip contaminated. When removing the column from the collection tube, be careful that the tip of the column does not come into contact with the flowthrough. Trace amounts of salt from the flowthrough can contaminate a sample resulting in a low A260/A230 ratio. Ethanol contamination from the flowthrough can also interfere with DNA elution. Zymo-Spin™ columns are designed for complete elution with no buffer retention or carryover.

Following Clean-up with the DCC™, Multiple Bands Appear in an Agarose Gel

• Acidification of DNA Loading Dye. Most loading dyes do not contain EDTA and will acidify (pH ≤ 4) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended.

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purified using the Zymoclean™ Gel DNA Recovery Kit is perfectly suited for use in DNA ligation reactions, sequencing, DNA labeling reactions, PCR, etc.

Gel purification from agarose gel

1 Excise the DNA fragment from the agarose gel using a razor blade, scalpel or other device and transfer it into a 1.5 ml microcentrifuge tube.

The amount of agarose excised from the gel should be as small as possible. Be sure to excise the desired band with very little or no extra agarose gel.

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2 Add 3 volumes of ADB to each volume of agarose excised from the gel (e.g., for 100 µl (mg) of agarose gel slice add 300 µl of ADB).

3 Incubate at 37-55 °C for 5-10 minutes until the gel slice is completely dissolved2. For DNA fragments > 8 kb, following the incubation step, add one additional volume (equal to that of the gel slice) of water to the mixture for better DNA recovery (e.g., 100 µl agarose, 300 µl ADB, and 100 µl water).

Do not incubate above 60°C. It is important that the gel slice dissolve completely. This can be facilitated by gentle mixing during the incubation.

4 Transfer the melted agarose solution to a Zymo-Spin™ Column in a provided Collection Tube.

5 Centrifuge for 30-60 seconds. Discard the flow-through

Remove the flow-through by aspiration. Avoid contamination of the collection tube rim.

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6 Add 200 µl of DNA Wash Buffer to the column and centrifuge for 30 seconds. Discard the flow-through. Repeat the wash step.

7 Add ≥ 6 µl DNA Elution Buffer or water directly to the column matrix. Place column into a 1.5 ml tube and centrifuge for 30-60 seconds to elute DNA. Ultra-pure DNA is now ready for use.

DNA Elution Buffer: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA.

Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. Waiting 1 minute prior to elution may improve the yield of larger (> 6 kb) DNA. For even larger DNA (> 10 kb), the total yield may be improved by eluting the DNA with 60-70°C DNA Elution Buffer.

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