Oct 02, 2020

DNA Clean & Concentrator-5--CHEM 584

DOI
dx.doi.org/10.17504/protocols.io.bmyyk7xw

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DOI: dx.doi.org/10.17504/protocols.io.bmyyk7xw

Protocol Citation: Ken Christensen 2020. DNA Clean & Concentrator-5--CHEM 584. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bmyyk7xw

Manuscript citation:
https://files.zymoresearch.com/protocols/_d4003t_d4003_d4004_d4013_d4014_dna_clean_concentrator_-5.pdf

Protocol status: In development
We are still developing and optimizing this protocol

Created: October 02, 2020

Last Modified: October 02, 2020

Protocol Integer ID: 42744

Abstract

Product Description

The DNA Clean & Concentrator™-5 (DCC™-5) provides a hassle-free method for the rapid purification and concentration of high-quality DNA from PCR, endonuclease digestions, cell lysates, and other impure DNA preparations. It can also be used for post-RT cDNA clean-up and purification of sequencing-ready DNA from M13 phage. Simply add the specially formulated DNA Binding Buffer to your sample and transfer the mixture to the supplied Zymo-Spin™ Column. There is no need for organic denaturants or chloroform. Instead, the product features Fast-Spin column technology to yield DNA that is free of salts and contaminants in just 2 minutes. The purified DNA is ideal for DNA ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.

Guidelines

Specifications

• DNA Purity – High-quality DNA (A260/A280 >1.8) ideal for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.

• 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 from enzymatic reactions (e.g., PCR, restriction endonuclease digestions), plasmid preparations, and impure preparations.

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

Troubleshooting

Low Recovery

• 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 and not to the walls of the column. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10 kb.

• 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) 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 Ratios

• 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 low A260/A230 ratios. 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.

PCR Cleanup/Cleanup after Restriction Digest

1 In a 1.5 ml microcentrifuge tube, add 2-7 volumes of DNA Binding Buffer to each volume of DNA sample (see table below). Mix briefly by vortexing.

Application	DNA Binding Buffer:Sample	Example
Plasmid, genomic DNA (>2 kb)	2:1	200 ul : 100 ul
PCR product, DNA fragment	5:1	500 ul : 100 ul
ssDNA	7:1	700 ul : 100 ul

2 Transfer mixture to a provided Zymo-Spin™ Column in a provided Collection Tube.

3 Centrifuge for 30 seconds. Discard the flow-through.

4 Add 200 µl DNA Wash Buffer to the column. Centrifuge for 30 seconds. Repeat the wash step.

5 Add ≥ 6 µl DNA Elution Buffer or water directly to the column matrix and incubate at room temperature for one minute. Transfer the column to a clean 1.5 ml microcentrifuge tube and centrifuge for 30 seconds to elute the DNA. Ultra-pure DNA is now ready for use.