Quick Protocol for Oligonucleotide Cleanup Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)

New England Biolabs

Abstract

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Guidelines

DNA Cleanup and Concentration: for the purification of up to 5 µg of DNA (ssDNA > 200 nt and dsDNA > 50 bp) from PCR and other enzymatic reactions. A detailed protocol and a quick protocol are available for your convenience.

Oligonucleotide Cleanup (steps): for the purification of up to 5 µg of DNA fragments ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA). Expected recovery is > 70%. When purifying ssDNA of any size, recovery can be increased by using this protocol; however, it is important to note that this protocol shifts the cutoff for smaller fragments to 18 nt (rather than 50 nt for the DNA Cleanup and Concentration Protocol).

Before start

- All centrifugation steps should be carried out at 16,000 x *g* (~13,000 RPM).
- Add 4 volumes of ethanol (≥ 95%) to one volume of DNA Wash Buffer.

Materials

Monarch® PCR & DNA Cleanup Kit (5 µg) T1030 by New England Biolabs

Protocol

Step 1.

Add 100 µl DNA Cleanup Binding Buffer to the 50 µl sample.

AMOUNT
100 µl Additional info: DNA Cleanup Binding Buffer

NOTES
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We recommend a sample volume of 50 µl. For smaller samples, adjust the volume with nuclease-free water.

Step 2.

Add 300 µl ethanol (≥ 95%). Mix well by pipetting up and down or flicking the tube. Do not vortex.

AMOUNT
300 µl Additional info: Ethanol (≥ 95%)

Step 3.

Insert column into collection tube and load sample onto column.

Step 4.

Spin for 1 minute, then discard flow-through.

Step 5.

Re-insert column into collection tube.

Step 6.

Add 500 µl DNA Wash Buffer and spin for 1 minute.

AMOUNT
500 µl Additional info: DNA Wash Buffer

Step 7.

Discard flow-through.

Step 8.

Repeat steps 5-7 (Optional). Recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K).

GOTO
Repeating steps 5-7 -> go to step #5

Step 9.

Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute.

Step 10.

Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix.

AMOUNT
6 µl Additional info: DNA Elution Buffer

Step 11.

Wait for 1 minute, then spin for 1 minute to elute DNA.

NOTES
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Typical elution volumes are 6–20 µl. Nuclease-free water (pH 7–8.5) can also be used to elute the DNA. Yield may slightly increase if a larger volume of DNA Elution Buffer is used, but the DNA will be less concentrated.

Warnings

Please refer to SDS for safety warnings.