Monarch® PCR & DNA Cleanup Kit (5 µg) Protocol Card

NEB #T1030

For a detailed protocol or to download the full manual, visit www.neb.com/T1030.

BEFORE YOU BEGIN:

- **IMPORTANT UPDATE:** Add 0.36 volumes of isopropanol to one volume of DNA Cleanup Binding Buffer (e.g., 63.5 ml isopropanol to 175 ml buffer)
- Add 4 volumes of ≥ 95% ethanol to one volume of DNA Wash Buffer (e.g., 100 ml ethanol to 25 ml buffer)
- All centrifugation steps should be carried out at 16,000 x *g* (~13,000 RPM)
- If working with DNA fragments ≥ 10 kb, preheat the appropriate amount of DNA Elution Buffer to 50°C

THERE ARE TWO PROTOCOLS AVAILABLE FOR THIS PRODUCT:

- **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
- **Oligonucleotide Cleanup:** 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).

DNA CLEANUP AND CONCENTRATION PROTOCOL STEPS:

1. **Dilute sample with DNA Cleanup Binding Buffer (ensure that isopropanol has been added, as indicated on the bottle label) according to the table below. Mix well by pipetting up and down or flicking the tube. Do not vortex.** We recommend a sample volume of 20-100 µl. For smaller samples, adjust the volume with TE. For diluted samples larger than 800 µl, load a portion of the sample, proceed with step 2, and repeat as necessary.

SAMPLE TYPE	RATIO OF BINDING BUFFER: SAMPLE	EXAMPLE
dsDNA > 2 kb (plasmids, gDNA)	2:1	200 µl: 100 µl
dsDNA < 2 kb (some amplicons, fragments)	5:1	500 µl: 100 µl
ssDNA* > 200 nt	7:1	700 µl: 100 µl

**Please note that recovery of ssDNA < 200 nts can be increased by using the Oligonucleotide Cleanup Protocol, but doing so will shift the cutoff size for DNA binding to 18 nt (versus 50 nt).*

2. **Insert column into collection tube and load sample onto column. Spin for 1 minute, then discard flow-through.**

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3. **Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute.** Discarding flow-through is optional.

4. **Repeat step 3.**

5. **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.

6. **Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.** 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. For larger size DNA (≥ 10 kb), heating the elution buffer to 50°C prior to use can improve yield.

Want to use this kit to purify DNA from agarose gels?
Simply purchase the Monarch Gel Dissolving Buffer (NEB #T1021L) and use with this kit. Protocol available at **www.neb.com/T1020**

Questions?
Our tech support scientists would be happy to help. Email us at **info@neb.com**

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OLIGONUCLEOTIDE CLEANUP PROTOCOL STEPS:

1. **Add 100 µl DNA Cleanup Binding Buffer (ensure that isopropanol has been added, as indicated on the bottle label) to the 50 µl sample.** We recommend a sample volume of 50 µl. For smaller samples, adjust the volume with nuclease-free water.

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

3. **Insert column into collection tube and load sample onto column. Spin for 1 minute, then discard flow-through.**

4. **Re-insert column into collection tube. Add 500 µl DNA Wash Buffer and spin for 1 minute.** Discard flow-through.

5. **(Optional) Repeat step 4.** This second wash step is optional, but recommended for removal of enzymes that may interfere with downstream applications (e.g., Proteinase K). Please note that if carrying out a second wash step, additional DNA Wash Buffer may be required.

6. **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.

7. **Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.** 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.

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