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© Quick Protocol for Monarch® DNA Gel Extraction Kit (NEB #T1020) V.3

New England Biolabs¹

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This is the quick version of the Monarch® DNA Gel Extraction Kit Protocol (NEB #T1020). For the full protocol, please click <u>here</u>.

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https://www.neb.com/protocols/2015/12/08/quick-protocol-for-monarch-dna-gel-extraction-kit-t1020

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Extracting, Monarch DNA Gel Extracting Kit, extracting dna, DNA, gel extraction

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For detailed protocol and more information, visit www.neb.com/T1020

The full protocol is available here.

A video protocol is available here.

MATERIALS

Monarch® DNA Gel Extraction Kit New England

Biolabs Catalog #T1020

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

- Add 4 volumes of ethanol (\geq 95%) to one volume of DNA Wash Buffer.
 - For 50-prep kit, add 20 ml of ethanol to 5 ml of Monarch DNA Wash Buffer
 - For 250-prep kit, add 100 ml of ethanol to 25 ml of Monarch DNA Wash Buffer
- All centrifugation steps should be carried out at 316.000 x g ($\sim \textcircled{313.000}$ rpm).
- Please note: column holds 800 μl
- 1 Excise the DNA fragment from the agarose gel, taking care to trim excess agarose.
 Transfer to a 1.5 ml microfuge tube and weigh the gel slice. Minimize exposure to UV light.
- 2 Add 4 volumes of Gel Dissolving Buffer to the gel slice (e.g., 400 μ l buffer per 100 μ l or 100 mg agarose).
- 3 Incubate the sample between 37–55°C (typically § 50 °C), vortexing periodically until the gel slice is completely dissolved (generally 5-10 minutes).

The time that takes a gel slice to melt depends on the size of the slice, the temperature used in the incubation as well as the percent agarose used in the gel. The time recommended above should be used just as a guideline.

For DNA fragments > 8 kb, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding of larger pieces of DNA (e.g., 100 μ l gel slice: 400 μ l Gel Dissolving Buffer: 150 μ l water).

Insert column into collection tube and load sample onto the column. Spin for

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- 5 Re-insert column into collection tube. Add

 □200 μL DNA Wash Buffer (with ethanol added) and spin for

 ③16000 x g, 00:01:00 . Discarding flow-through is optional.
- 6 Repeat Step 5: Re-insert column into collection tube. Add

 200 μL DNA Wash Buffer (with ethanol added) and spin for

 316000 x g, 00:01:00. Discarding flow-through is optional.
- 7 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 ③ 00:01:00.
- 8 Add $\geq \frac{1}{2}6 \,\mu\text{L}$ DNA Elution Buffer to the center of the matrix. Wait for $\circlearrowleft 00:01:00$, and spin for $\circledcirc 16000 \, x \, g$, 00:01:00 to elute the DNA.

Typical elution volumes are $6-20 \,\mu$ l. 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 ($\geq 10 \, \text{kb}$), heating the elution buffer to 50° C prior to use can improve yield.