Quick Protocol for Monarch® DNA Gel Extraction Kit (NEB #T1020)

New England Biolabs

Abstract

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

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Guidelines

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

The full protocol is available here.

A video protocol is available here.

Before start

Add 4 volumes of ethanol (≥ 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 16,000 x g (~13,000 RPM).

Please note: column holds 800 µl

Materials

Monarch® DNA Gel Extraction Kit T1020 by New England Biolabs

Protocol

Step 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.

NOTES

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Trim/remove excess agarose and minimize exposure to UV light.

Step 2.
Add 4 volumes of Gel Dissolving Buffer to the gel slice (e.g., 400 µl buffer per 100 µl or 100 mg agarose).

Step 3.
Incubate the sample between 37–55°C (typically 50°C), 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.

NOTES
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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 µl gel slice: 400 µl Gel Dissolving Buffer: 150 µl water).

Step 4.
Insert column into collection tube and load sample onto the column. Spin for 1 minute at 16,000 x g, then discard flow-through.

DURATION
00:01:00

Step 5.
Re-insert column into collection tube. Add 200 µl DNA Wash Buffer (with ethanol added) and spin for 1 minute at 16,000 x g. Discarding flow-through is optional.

DURATION
00:01:00

Step 6.
Repeat Step 5 (Step 5: Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute at 16,000 x g. Discarding flow-through is optional).

DURATION
00:01:00

Step 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 1 minute.

NOTES
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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 8.
Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, and spin for 1 minute at 16,000 x g to elute the DNA.

DURATION
00:02:00

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. For larger size DNA (≥ 10 kb), heating the elution buffer to 50°C prior to use can improve yield.