Version 3

Nov 30, 2020

Quick Protocol for Monarch® Plasmid Miniprep Kit (NEB #T1010) V.3

New England Biolabs[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.bp9fmr3n

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ABSTRACT

This is the quick version of the Monarch® Plasmid DNA Miniprep Kit Protocol (NEB #T1010). For the full protocol, please click here.

EXTERNAL LINK

https://www.neb.com/protocols/2015/12/08/quick-protocol-for-monarch-plasmid-miniprep-kit-t1010

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PROTOCOL CITATION

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EXTERNAL LINK

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GUIDELINES

For detailed protocol and more information, visit **www.neb.com/T1010**

The full protocol is available here.

The video protocol is available here.

MATERIALS TEXT

MATERIALS

Monarch® Plasmid Miniprep Kit **New England Biolabs Catalog #T1010**

ABSTRACT

This is the quick version of the Monarch® Plasmid DNA Miniprep Kit Protocol (NEB #T1010). For the full protocol, please click here.

BEFORE STARTING

- Please review the important information under the “Guidelines" tab before beginning.
- 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 Plasmid Wash Buffer 2.
- If precipitate has formed in Lysis Buffer (B2), incubate at 30–37°C, inverting periodically to dissolve.
- Store Plasmid Neutralization Buffer (B3) at 4°C after opening, as it contains RNase A.

1 Pellet 1–5 ml (not to exceed 15 OD units) bacterial culture by centrifugation for 30 seconds. Discard supernatant.

00:00:30

1.5 ml of culture is sufficient for most applications. Ensure cultures are not overgrown (12-16 hours is ideal).

2 Resuspend pellet in 200 µl Plasmid Resuspension Buffer (B1).

Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.

3 Add 200 µl Plasmid Lysis Buffer (B2), gently invert tube 5–6 times, and incubate at room temperature for 1 minute. Do not vortex.

00:01:00 incubation

Color should change to dark pink, and solution will become transparent and viscous. Do not vortex.

4 Add 400 µl of Plasmid Neutralization Buffer (B3), gently invert tube until neutralized, and incubate at room temperature

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for 1 minute. Do not vortex.

00:02:00 incubation

Sample is neutralized when color is uniformly yellow and precipitate forms. Do not vortex.

5 Centrifuge lysate for 2–5 minutes.

00:02:00 centrifugation

For culture volumes >1 ml, we recommend a 5 minute spin to ensure efficient RNA removal by RNase A. Pellet should be compact; spin longer if needed.

6 Carefully transfer supernatant to the spin column and centrifuge for 1 minute. Discard flow-through.

00:01:00 centrifugation

7 Re-insert column in the collection tube and add 200 µl of Plasmid Wash Buffer 1. Centrifuge for 1 minute.

00:01:00

Discarding the flow-through is optional.

8 Add 400 µl of Plasmid Wash Buffer 2 and centrifuge for 1 minute.

00:01:00 centrifugation

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 there is any doubt, re-spin the column for 1 minute.

10 Add ≥ 30 µl DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.

00:01:00 wait

00:01:00 spin

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.

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