Quick Protocol for Monarch® Plasmid Miniprep Kit (NEB #T1010) Version 2

New England Biolabs

Abstract

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

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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.

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 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.

Materials

Monarch® Plasmid Miniprep Kit T1010 by New England Biolabs

Protocol

Step 1.

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

DURATION
00:00:30

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

Step 2.

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

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

Step 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.

DURATION
00:01:00

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

Step 4.

Add 400 µl of Plasmid Neutralization Buffer (B3), gently invert tube until neutralized, and incubate at room temperature for 1 minute. Do not vortex.

DURATION
00:02:00

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

Step 5.

Centrifuge lysate for 2–5 minutes.

DURATION
00:02:00

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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.

Step 6.

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

DURATION
00:01:00

Step 7.

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

DURATION
00:01:00

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Discarding the flow-through is optional.

Step 8.

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

DURATION
00:01:00

Step 9.

Transfer column to a clean 1.5 ml microfuge tube.

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

Step 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.

NOTES
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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.