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

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

- For 50-prep kit add 24 ml of ethanol to 6 ml of Monarch Plasmid Wash Buffer 2
- For 250-prep kit add 144 ml of ethanol to 36 ml of Monarch Plasmid Wash Buffer 2

Add 4 volumes of ethanol (≥ 95%) to one volume of Plasmid Wash Buffer 2.

All centrifugation steps should be carried out at 16,000 x g (~13,000 RPM).

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.

Materials

Monarch® Plasmid Miniprep Kit T1010 by New England Biolabs

Protocol

Step 1.

Pellet 1–5 ml bacterial culture by centrifugation at 16,000 x g for 30 seconds. Discard supernatant.

DURATION
00:00:30

Step 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.

Step 3.
Add 200 µl Plasmid Lysis Buffer (B2), gently invert tube 5–6 times, and incubate at room temperature for 1 minute. Color should change to dark pink, and solution will become transparent and viscous. 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 2 minutes. Sample is neutralized when color is uniformly yellow and precipitate forms. 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 at 16,000 x g for 2–5 minutes. 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.

DURATION
00:02:00

Step 6.
Carefully transfer supernatant to the spin column and centrifuge at 16,000 x g 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 at 16,000 x g. Discarding the flow-through is optional.

DURATION
00:01:00

Step 8.
Add 400 µl of Plasmid Wash Buffer 2 and centrifuge at 16,000 x g for 1 minute.

DURATION
00:01:00

Step 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.

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 at 16,000 x g to elute the DNA. Nuclease-free water (pH 7–8.5) can also be used to elute the DNA.

DURATION

00:02:00

ANNOTATIONS

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