Monarch® Plasmid Miniprep Kit Protocol Card

NEB #T1010

For a detailed protocol or to download the full manual, visit www.neb.com/T1010.

BEFORE YOU BEGIN:

- 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, as it contains RNase A.
- If working with plasmids ≥ 10 kb, preheat the appropriate amount of DNA Elution Buffer to 50°C.

PROTOCOL STEPS:

1. **Pellet 1–5 ml (not to exceed 15 OD units) bacterial culture by centrifugation for 30 seconds. Discard supernatant.** 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.** 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 for 2 minutes.** Sample is neutralized when color is uniformly yellow and precipitate forms. Do not vortex.
5. **Centrifuge lysate for 2–5 minutes.** For best results, and especially 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.

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

7. **Re-insert column in the collection tube and add 200 µl of Plasmid Wash Buffer 1. Centrifuge for 1 minute.** Discarding the flow-through is optional.

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

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.** 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.

Questions?
Our tech support scientists would be happy to help.
Email us at **info@neb.com**

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