NEW ENGLAND Biolabs®

Plasmid Miniprep Protocol using Centrifugation (NEB #T1110)

Materials Required but not Supplied

Monarch® Spin Plasmid Miniprep Kit

- Isopropanol (≥ 99%)
- Ethanol (≥ 95%)
- Nuclease-free Water (NEB #B1500)

Overview

Buffer Preparation

Prepare buffers as recommended in Buffer Preparation Guidance.

Plasmid Miniprep Protocol

1. **Pellet 1–5 ml bacterial culture (not to exceed 15 OD units) by centrifugation for 30 seconds. Discard the supernatant.** For a standard miniprep to prepare plasmid for restriction digestion or PCR, we recommend 1.5 ml of culture, which is sufficient for most applications. Ensure cultures are not overgrown; 12–16 hours is usually ideal for optimal growth.

2. **Resuspend the pellet in 200 µl of Monarch Buffer B1 (pink ●).** Vortex or pipet mix to ensure cells are completely resuspended. There should be no visible clumps.

3. **Add 200 µl of Monarch Buffer B2 (blue ●), gently invert the tube 5-6 times, and incubate at room temperature for 1 minute. Do not vortex.** The color should change to dark pink, and the solution should be transparent and viscous. Handle the sample gently to reduce the risk of shearing chromosomal DNA, which can be co-purified as a contaminant. Avoid incubating longer than one minute to prevent irreversible plasmid denaturation.

4. **Add 400 µl of Monarch Buffer B3 (yellow ●), and gently invert the tube until neutralized. Do not vortex.** The color should be uniformly yellow and a precipitate will form. Incubate for 2 minutes. Gentle but uniform mixing will ensure complete neutralization without shearing chromosomal DNA.

5. **Centrifuge the lysate for 2–5 minutes.** The pellet should be compact; spin longer if needed. Spin time should not be less than 2 minutes. For culture volumes > 1 ml, we recommend a longer spin (~5 minutes) to ensure efficient RNA removal by RNase A and a more compact pellet, which will lower the risk of clogging the column.

6. **Carefully transfer the supernatant to the Monarch Spin Column S2D and centrifuge for 1 minute. Discard the flow-through.**

7. **Re-insert the Monarch Spin Column S2D in the Monarch Spin Collection Tube and add 200 µl of Monarch Buffer BZ (wash 1). Centrifuge for 1 minute.** Discarding the flow-through is optional. This is a high-salt wash step that helps remove any residual RNA, protein, and other contaminants. Incubate for 5 minutes after adding Monarch Buffer BZ and before centrifugation if the plasmid will be used for transfection.

8. **Wash by adding 400 µl of Monarch Buffer WZ (wash 2) and centrifuge for 1 minute.**

9. **Transfer the column to a clean 1.5 ml microfuge tube.** Use care to ensure that the tip of the column does not touch the flow-through. If in doubt, re-spin for 1 minute.

10. **Add ≥ 30 µl of Monarch Buffer EY 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 Monarch Buffer EY is used, but the DNA will be less concentrated. For larger size plasmids (≥ 15 kb), incubate the column with elution buffer at room temperature for 5 minutes to maximize the yield. Alternatively, the elution buffer can be heated to 50°C before use.