Monarch® DNA Gel Extraction Kit Protocol Card

NEB #T1020

For a detailed protocol, or to download the full manual, visit www.neb.com/T1020.

BEFORE YOU BEGIN:

- Add 4 volumes of ethanol (≥ 95%) per volume of DNA Wash Buffer.
- All centrifugation steps should be carried out at 16,000 x *g* (~13,000 RPM).
- If working with DNA fragments ≥ 10 kb, preheat the appropriate amount of DNA Elution Buffer to 50°C.
- Please note that the column reservoir holds 800 µl.

PROTOCOL STEPS:

1. **Excise the DNA fragment from the agarose gel, taking care to trim excess agarose. Transfer to a 1.5 ml microfuge tube, and weigh the gel slice.** Minimize exposure to UV light.
2. **Add 4 volumes of Gel Dissolving Buffer to the gel slice** (e.g., 400 µl buffer per 100 mg agarose). If the gel slice is > 150 mg, consider reducing the amount of Gel Dissolving Buffer to 3 or 3.5 volumes to minimize the guanidine salt present in the workflow.
3. **Incubate the sample between 37-55°C (typically 50°C), inverting periodically until the gel slice is completely dissolved** (generally 5-10 minutes). For DNA fragments > 8 kb, an additional 1.5 volumes of water should be added after the slice is dissolved to mitigate the tighter binding of larger pieces of DNA (e.g., 100 µl gel slice: 400 µl Gel Dissolving Buffer: 150 µl water).
4. **Insert column into collection tube and load sample onto the column. Spin for 1 minute, then discard flow-through.**
5. **Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute.** Discarding flow-through is optional.
6. **Repeat step 5.**

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7. **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 in doubt, re-spin for 1 minute.

8. **Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, and spin for 1 minute to elute DNA.** Typical elution volumes are 6-20 µl. 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.

Want to use this kit to purify DNA from PCR and other enzymatic reactions?
Simply purchase the Monarch DNA Cleanup Binding Buffer (NEB #T1031L) and use with this kit. Protocol available at **www.neb.com/T1030**

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

V5.0 – 1.21 #101028

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