

Protocol for Monarch Genomic DNA Extraction (NEB #T3010)

Part 1: Sample Preparation - Genomic DNA Cleanup

Overview

There are two protocols provided for the cleanup of genomic DNA. The Desalting/Buffer Exchange Cleanup Protocol is for cleanup of salts and buffer components. The Enzymatic Cleanup Protocol should be employed if the removal of proteins and/or RNA is necessary.

Before You Begin:

- Store RNase A and Proteinase K at -20°C.
- Add ethanol ($\geq 95\%$) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Set a thermal mixer (e.g. ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- Set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 μl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Genomic DNA Extraction Consists of Two Stages:

PART 1: Sample Preparation

PART 2: **Genomic DNA Binding and Elution**

Desalting/Buffer Exchange Cleanup Protocol

1. Add DNA sample to a 1.5 ml reaction tube and bring the volume up to 200 μl with nuclease-free water. Mix well by vortexing. If the total DNA input amount is less than 100 ng add 10 $\mu\text{g}/\text{ml}$ of carrier RNA to the gDNA Binding Buffer for quantitative retrieval of the DNA (See “Use of Carrier RNA for Low Input Amounts”).
2. **Proceed to Part 2: Genomic DNA Binding and Elution.**

Enzymatic Cleanup Protocol (removal of proteins and/or RNA)

1. Add DNA sample to a 1.5 ml reaction tube and bring the volume up to 200 μl with Tissue Lysis Buffer. Mix well by vortexing.

2. Add 1 μ l of Proteinase K and, if RNA needs to be removed, add 1 μ l RNase A.
3. Mix briefly and incubate at 56°C for 5 minutes.
4. [Proceed to Part 2: Genomic DNA Binding and Elution.](#)

Additional Resources you may find helpful:

- [Monarch Spin gDNA Extraction Kit Product Manual](#)
- [Choosing Input Amounts for the Monarch Spin gDNA Extraction Kit](#)
- [Troubleshooting Guide for Genomic DNA Extraction & Purification](#)
- [Factors Affecting DNA Quality when Purifying gDNA from Blood and Tissues with the Monarch Spin gDNA Extraction Kit](#)