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 (≥ 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 µg/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 µl of Proteinase K and, if RNA needs to be removed, add 1 µl 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