Genomic DNA Purification from Gram-negative Bacteria (NEB #T3010)

Up to 2 x 10^9 Gram-negative bacteria can be processed using either a quick protocol which employs Lysozyme for bacterial cell wall lysis, or a longer protocol that does not require enzymatic lysis with Lysozyme. Both protocols are available below.

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.
- Cold PBS or 10 mM Tris-HCl pH 8.0 is required (not supplied).
- Set a thermal mixer (e.g. ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- For Lysozyme-based Lysis:
  - Set a thermal mixer or heating block to 37°C.
  - Prepare or thaw a stock solution of Lysozyme (not supplied) (25 mg/ml in water or 10 mM Tris-Cl, pH 8.0)
- To prepare for elution, 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.

Rapid Protocol (requires Lysozyme)

1. Harvest a maximum of up to 2 x 10^9 Gram-negative bacteria by centrifugation for 1 minute at > 12,000 x g. Discard supernatant.
2. Add 90 µl of cold PBS or 10 mM Tris-Cl pH 8.0 and resuspend bacterial pellet by vortexing.
3. Add 10 µl Lysozyme solution (25 mg/ml) and vortex briefly, then add 100 µl Tissue Lysis Buffer and vortex thoroughly.
4. Incubate at 37°C for 5 minutes or until clear. Most lysates will become fully clear, but for some bacteria a slight haze may remain.
5. Add 10 µl Proteinase K, vortex briefly, and incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at full speed (~1400 rpm).
6. Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed (~1400 rpm).
7. Proceed to Genomic DNA Binding and Elution.

Simplified Protocol (no Lysozyme required)

1. Harvest a maximum of up to 2 x 10^9 Gram-negative bacteria by centrifugation for 1 minute at > 12,000 x g. Remove supernatant.
2. Add 100 µl of PBS or 10 mM Tris-Cl pH 8.0 and resuspend bacterial pellet by vortexing.
3. Add 10 µl Proteinase K and vortex briefly, then add 100 µl Tissue Lysis Buffer and vortex thoroughly.
4. Incubate at 56°C for 1–4 hours in a thermal mixer with agitation at full speed until the lysate is mostly clear and ceases to change in appearance (lysis is usually complete within 2 hours).
5. Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed (~1400 rpm).
6. Proceed to Genomic DNA Binding and Elution.

GENOMIC DNA BINDING AND ELUTION

1. **Add 400 µl gDNA Binding Buffer to the sample and mix thoroughly by pulse-vortexing for 5-10 seconds.** Thorough mixing is essential for optimal results.
2. **Transfer the lysate/binding buffer mix (~600 µl) to a gDNA Purification Column pre-inserted into a collection tube, without touching the upper column area. Proceed immediately to step 3.** Do not reload the same column with more sample; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge. Avoid touching the upper column area with lysate/binding mix and avoid transferring foam that may have formed during lysis. Any material that touches the upper area of the column, including any foam, may lead to salt contamination in the eluate.
3. **Close the cap and centrifuge: first for 3 minutes at 1,000 x *g* to bind gDNA (no need to empty the collection tubes or remove from centrifuge) and then for 1 minute at maximum speed (> 12,000 x *g*) to clear the membrane. Discard the flow-through and the collection tube.** For optimal results, ensure that the spin column is placed in the centrifuge in the same orientation at each spin step (for example, always with the hinge pointing to the outside of the centrifuge); ensuring the liquid follows the same path through the membrane for binding and elution can slightly improve yield and consistencv.

4. **Transfer column to a new collection tube and add 500 µl gDNA Wash Buffer. Close the cap and invert a few times, so that the wash buffer reaches the cap. Centrifuge immediately for 1 minute at maximum speed (12,000 x *g*), and discard the flow through.** The collection tube can be tapped on a paper towel to remove any residual buffer before reusing it in the next step. Inverting the spin column containing wash buffer prevents salt contamination in the eluate.

5. **Reinsert the column into the collection tube. Add 500 µl gDNA Wash Buffer and close the cap. Centrifuge immediately for 1 minute at maximum speed (>12,000 x *g*), then discard the collection tube and flow through.**

6. **Place the gDNA Purification Column in a DNase-free 1.5 ml microfuge tube (not included). Add 35-100 µl preheated (60°C) gDNA Elution Buffer, close the cap and incubate at room temperature for 1 minute.** Elution in 100 µl is recommended, but smaller volumes can be used and will result in more concentrated DNA but a reduced yield (20–25% reduction when using 35 µl). Eluting with preheated elution buffer will increase yields by ~20–40% and eliminates the need for a second elution. For applications in which a high DNA concentration is required, using a small elution volume and then eluting again with the eluate may increase yield (~10%). The elution buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA) offers strong protection against enzymatic degradation and is optimal for long term storage of DNA. However, other low-salt buffers or nuclease-free water can be used if preferred. For more details on optimizing elution, please refer to “Considerations for Elution & Storage” in the product manual.

7. **Centrifuge for 1 minute at maximum speed (> 12,000 x *g*) to elute the gDNA.**

Additional Resources you may find helpful:

- Monarch Genomic DNA Purification Kit Product Manual
- Choosing Input Amounts for the Monarch Genomic DNA Purification Kit
- Troubleshooting Guide for Genomic DNA Extraction & Purification
- Factors Affecting DNA Quality when Purifying gDNA from Blood and Tissues with the Monarch Genomic DNA Purification Kit
- Guidelines for Handling Tissue Samples when using the Monarch Genomic DNA Purification Kit

Links to this resource

Product Categories: Genomic DNA Extraction & Purification, Nucleic Acid Purification Products

Related Products: Monarch® Genomic DNA Purification Kit, T3018 Monarch RNase A, T3013 Monarch gDNA Blood Lysis Buffer, | More +

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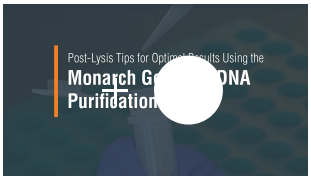

Post-lysis Tips for Optimal Results using the Monarch® Genomic DNA Purification Kit

After lysing your sample during your DNA extraction workflow, follow these tips to ensure that your purified gDNA is high-quality, intact, and free from contaminants.