Monarch® HMW DNA Extraction Kit: Tissue Protocol

NEB #T3060

We strongly recommend that first-time users read the product manual at www.neb.com/T3060 and review the entire protocol before beginning. The product manual provides additional commentary and considerations for various steps, as well as guidance on tissue input amounts, handling, and homogenization options. This shortened protocol is meant for experienced users. Supplemental protocols, including HMW DNA extraction from bacteria, can be found in the product manual in our online resources, which can be accessed using the QR code to the right.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Microcentrifuge
- Thermal mixer containing a 1.5 ml block (optional: 2 ml block)
- Isopropanol
- Ethanol (≥ 95%)
- Vertical rotating mixer
- 1.5 ml microfuge tubes (DNase-free, DNA low bind recommended)
- Wide-bore pipette tips
- Optional: rotor-stator homogenizer (e.g., TissueRuptor® II), and compatible 2 ml tubes

IMPORTANT NOTES BEFORE YOU BEGIN

- ❑ Store RNase A and Proteinase K at -20°C upon opening the kit.
- ❑ Add ethanol (≥ 95%) to the gDNA Wash Buffer concentrate as indicated on the bottle label.
- ❑ Preheat thermal mixer with 1.5 ml block to 56°C.

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INPUT GUIDANCE	
PROTOCOL DESIGNATION	**TISSUE AMOUNT**
Standard Input	10-25 mg for most tissues 5-15 mg for soft organ/DNA-rich tissues (e.g., liver*) 10-20 mg for fatty tissues (e.g., brain)
Low Input	5- < 10 mg for most tissues** 2- < 5 mg for soft organ/DNA-rich tissue

* For guidance on processing liver samples, refer to the product manual.
** For brain and muscle samples < 5 mg, refer to the product manual.

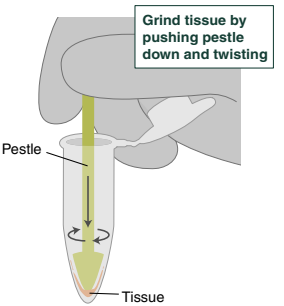
Part 1: TISSUE LYSIS

1. **Prepare a master mix of HMW gDNA Tissue Lysis Buffer and Proteinase K according to the table below and the number of samples that will be processed.**

PROTOCOL DESIGNATION	VOLUME OF HMW gDNA TISSUE LYSIS BUFFER (µl)	VOLUME OF PROTEINASE K (µl)
Standard Input	600 µl	20 µl
Low Input	300 µl	10 µl

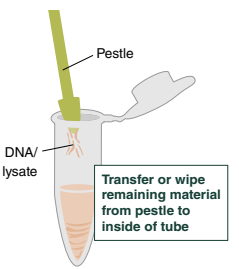
2. **Transfer the desired amount of tissue to a Monarch Pestle Tube (or, if using a rotor-stator homogenizer, to a compatible 2 ml tube). Place on ice (fresh samples) or dry ice (frozen samples).**
3. **Briefly spin sample (2-3 seconds) in a benchtop minicentrifuge to collect all tissue material at the bottom of the tube.** For frozen samples, thaw each sample at room temp, spin down, and proceed quickly to homogenization. Then, repeat for any remaining samples.
4. **Homogenization can be carried out using one of two methods: using a pestle or rotor-stator homogenizer. If working with multiple samples, each sample should be taken quickly through all sub-steps and placed in the thermal mixer before processing the next sample.** For guidance on using a rotor stator homogenizer, please refer to the product manual.

a. **Use the pestle to grind the sample within the pestle tube; leave the pestle in the tube.**



b. **Using a wide bore pipette tip, add 600 µl (Low Input: 300 µl) of the lysis master mix to the sample.** Do not dispose of this tip yet, as it will be used to mix the sample.

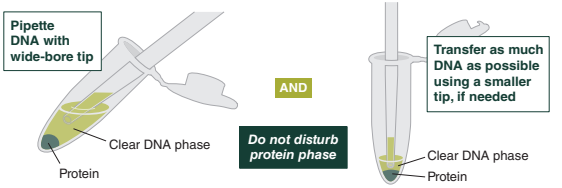
c. **Ensure there is no tissue material remaining on the pestle, then discard the pestle.** If tissue material sticks to the pestle, transfer it carefully into the tube.



d. **Using the wide-bore tip, pipette up and down a few times to homogenize the tissue lysate to ensure rapid, complete lysis.** Discard the pipette tip.

e. **Begin incubation in the thermal mixer (Step 5), and repeat steps a-e with any remaining samples.**

5. **Incubate at 56°C for 45 minutes in a thermal mixer with agitation at the desired speed.** If working with input amounts near the lower end of the input range: stop agitation after 15 minutes, and finish the incubation without shaking to maximize yield. Refer to the product manual for guidance on choosing an agitation speed; higher speeds reduce DNA frament size. For standard ligation-based nanopore sequencing, 2,000 rpm is recommended. For the largest DNA, use 500-700 rpm. Optimization may be required depending on the quality of the starting material. If desired, samples can be stored at 4°C overnight after this incubation.
6. **Add 10 µl (Low Input: 5 µl) RNase A and mix by inverting 5-10 times. Incubate for 10 minutes at 56°C at the same agitation speed used in Step 5.** For fatty tissues (e.g., brain), place on ice for 3 minutes after incubation.
7. **Change the heat block in the thermal mixer to accommodate a 2 ml tube, and preheat the block to 56°C.** If a 2 ml heat block is not available, continue working with the 1.5 ml block.
8. **Add 300 µl (Low Input: 150 µl) of Protein Separation Solution and mix by inverting for 1 minute.** Alternatively, a vertical rotating mixer at 20 rpm can be used.
9. **Centrifuge for 10 minutes at 16,000 x *g*. If working with multiple samples, during centrifugation, prepare the plastics for Part 2, as indicated in the following step.** The sample will separate into a large, clear, upper phase (DNA) and a smaller (often brown) protein phase, usually on the bottom of the tube. For some tissues, the protein phase may be yellow or clear. Additional centrifugation time (10-20 minutes) may be required for complete phase separation, particularly when low agitation speeds were used.
10. **If working with multiple samples, prepare and label the plastics for the upcoming steps.** Each sample will require (1) Monarch Collection Tube II, (1) Monarch Bead Retainer, (2) Monarch 2 ml Tubes, (1) microfuge tube (1.5 ml, DNA low bind recommended, not provided).
11. **Using a 1000 µl (Low Input: 200 µl) wide-bore pipette tip, transfer the upper phase containing the DNA (large, clear phase) to a labeled Monarch 2 ml Tube.** Highest yields will be achieved by transferring as much of the upper phase as possible. Using a 200 µl wide-bore pipette tip to transfer the final volume of upper phase is recommended for maximum yield. Avoid transferring material from the protein layer, although a small amount (1-2 µl) will not be detrimental. If protein enters the pipette tip, gently push it back into the tube. If a lower protein phase is not visible, leave ~30 µl behind to ensure protein is not carried over.



Part 2: HMW gDNA BINDING AND ELUTION

1. **Using clean forceps, add 2 DNA Capture Beads to each sample, which should be contained in a Monarch 2 ml Tube.**
2. **Add 550 µl (Low Input: 275 µl) isopropanol, close the cap, and mix on a vertical rotating mixer at 10 rpm for 5 minutes to attach DNA to the beads.** If a vertical rotating mixer is not available, invert slowly and gently by hand 25-30 times. A manual inversion is complete when the tube returns to the upright position. Slow inversion is critical for the DNA to bind to the beads; each full inversion should take ~5-6 seconds.
3. **Remove liquid by pipetting. Avoid removing any of the gDNA wrapped around the glass beads. For optimal DNA solubility, avoid letting the bound DNA dry out on the beads during this and the following steps; add the next buffer quickly.** Keeping tube upright, insert pipette tip and gently push beads aside to remove liquid, or tilt the tube almost horizontally and remove liquid from the top of the angled tube.

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4. **Add 500 µl gDNA Wash Buffer, close the cap and mix by inverting the tube 2-3 times. Remove the wash buffer as described in the previous step.**
5. **Repeat the wash in Step 4, and remove the wash buffer by pipetting.** Alternatively, the buffer can be removed by decanting: position a pipette tip at the top of the angled tube to prevent the beads from falling out. It is not necessary to remove all the gDNA Wash Buffer at this point.
6. **Place a labeled bead retainer into a Monarch Collection Tube II. Pour the beads into the bead retainer and close the cap. Discard the used Monarch 2 ml Tube.** If working with multiple samples, be sure to close the cap after each bead transfer.
7. **Pulse spin (≤ 1 second) the sample in a benchtop minicentrifuge to remove residual wash buffer from the beads.**
8. **Separate the bead retainer from the collection tube, pour the beads into a new, labeled Monarch 2 ml Tube, and insert the used bead retainer into a labeled 1.5 ml microfuge tube (DNA low bind recommended, not provided). Discard the used collection tube.**
9. **Immediately add 100 µl Elution Buffer II onto the glass beads and incubate for 5 minutes at 56°C in a thermal mixer with agitation at 300 rpm.** Halfway through the incubation, ensure beads are not stuck by tilting the tube almost horizontally and gently shaking; do not let liquid reach the cap and avoid splashing.
10. **Ensure bead retainer is inserted into the 1.5 ml microfuge tube. Pour eluate and beads into the bead retainer and close the cap.**
11. **Centrifuge for 30 seconds at 12,000 x *g* to separate the eluate from the glass beads. Discard the beads and retainer.**
12. **Pipette eluate up and down 5-10 times with a wide bore pipette tip and ensure any visible DNA aggregates are dispersed.** See product manual for guidance on preparing DNA for downstream use. Samples can be stored at 4°C for short term use (weeks) or -20°C for long term storage. The elution buffer (10 mM Tris, pH 9.0, 0.5 mM EDTA) is formulated for long term storage of gDNA.

Note: DNA fragment length from pestle-homogenized tissue samples agitated at 2000 rpm often ranges up to 500 kb. As the optimal fragment length for standard ligation-based nanopore sequencing is 50-250 kb, the eluate can be passed through a 26-gauge blunt-end needle 10-20 times to reduce the fragment size.

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

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