

Monarch[®] HMW DNA Extraction Kit: Cell Protocol

NEB #T3050

We strongly recommend that first-time users read the product manual at www.neb.com/T3050 and review the entire protocol before beginning. The product manual provides additional commentary and considerations for various steps. This shortened protocol is meant for experienced users. Access the manual, protocols and other resources using the QR code to the right.



MATERIALS REQUIRED BUT NOT SUPPLIED

- Microcentrifuge
- Thermal mixer containing a 2 ml block (1.5 ml block can also be used)
- Ethanol ($\geq 95\%$)
- Isopropanol
- 1.5 ml microfuge tubes (DNase-free, DNA low bind recommended)
- Vertical rotating mixer
- Wide-bore pipette tips

IMPORTANT NOTES BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C and RBC Lysis Buffer at 4°C upon opening the kit.
- Add ethanol ($\geq 95\%$) to the gDNA Wash Buffer concentrate as indicated on the bottle label.
- Cool Nuclei Prep Buffer to 4°C ; store at 4°C for convenience.
- Preheat thermal mixer with 2 ml block to 56°C .

Use the table below to determine the designation of your sample type, which will determine various volumes in the protocol.

PROTOCOL DESIGNATION	NUMBER OF CELLS
Standard Input	> 5×10^5 – 1×10^7 ; 1×10^6 is recommended Do not exceed 5×10^6 cells if using 300 rpm agitation for "XL DNA"
Low Input	1×10^5 – $\leq 5 \times 10^5$ Below 1×10^5 cells, DNA recovery is significantly less efficient.

Part 1: CELL LYSIS

- Pellet cells in a Monarch 2 ml Tube by centrifugation for 3 minutes at 1,000 x g. Frozen pellets should be thawed.
- Prepare Nuclei Prep and Lysis Solutions as indicated below:
 - Nuclei Prep Solution:** combine cold Nuclei Prep Buffer and RNase A according to the following table and vortex to mix. Keep on ice.

# OF SAMPLES	STANDARD INPUT		LOW INPUT	
	VOLUME OF NUCLEI PREP BUFFER (μl)	VOLUME OF RNASE A (μl)	VOLUME OF NUCLEI PREP BUFFER (μl)	VOLUME OF RNASE A (μl)
1	165	5.5	55	2
2	330	11	110	4
3	495	16.5	165	6
4	660	22	220	8
5	825	27.5	275	10

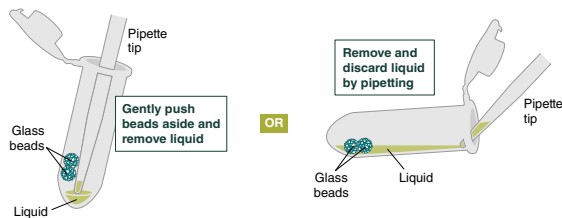
- Nuclei Lysis Solution:** combine Nuclei Lysis Buffer and Proteinase K according to the following table and vortex to mix. Keep at room temperature.

# OF SAMPLES	STANDARD INPUT		LOW INPUT	
	VOLUME OF NUCLEI LYSIS BUFFER (μl)	VOLUME OF PROTEINASE K (μl)	VOLUME OF NUCLEI LYSIS BUFFER (μl)	VOLUME OF PROTEINASE K (μl)
1	165	11	55	4
2	330	22	110	8
3	495	33	165	12
4	660	44	220	16
5	825	55	275	20

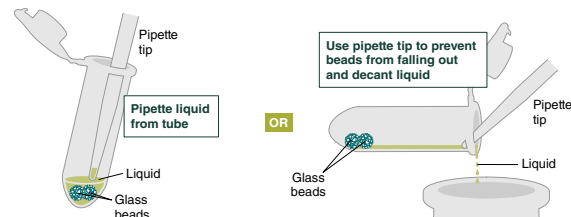
- Flick to resuspend cell pellet. Add 150 μl (**Low Input: 50 μl**) of Nuclei Prep Solution and pipette up and down 10 times to mix, being careful not to introduce air bubbles. Incubate at room temperature for 2 minutes. The sample will become less turbid, indicating cell lysis; nuclei remain intact.
- Add 150 μl (**Low Input: 50 μl**) of Nuclei Lysis Solution to sample and invert 10 times to mix. Do not vortex or pipette.
- Incubate at 56°C for 10 minutes in a thermal mixer with agitation at the desired speed to control the shearing and tune the size of gDNA. The speed of the thermal mixer influences fragment length; higher speeds reduce overall size. For standard ligation-based nanopore sequencing, 2,000 rpm is recommended. For the largest DNA, use 300 rpm. Optimization may be required depending on the quality of the starting sample. Refer to "Choosing Agitation Speed During Lysis" in the product manual for guidance. If desired, samples can be stored at 4°C overnight after this incubation.
- If working with multiple samples, prepare and label the plastics for Part 2. Each sample will require (1) Monarch Collection Tube II, (1) Monarch Bead Retainer, (1) Monarch 2 ml Tube, (1) 1.5 ml microfuge tube (DNA low bind recommended, not provided).
- Add 75 μl (**Low Input: 25 μl**) Precipitation Enhancer and mix by inverting 8-10 times. Proceed to Part 2: HMW gDNA Binding and Elution.

Part 2: HMW gDNA BINDING AND ELUTION

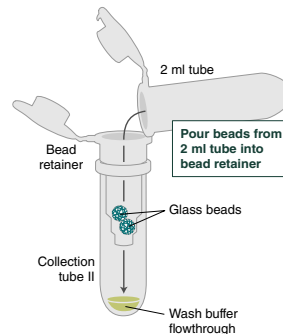
1. Using clean forceps, add 2 DNA Capture Beads to each sample, which should be contained in a Monarch 2 ml Tube.
2. Add 275 μl (Low Input: 100 μl) isopropanol, close the cap, and mix on a vertical rotating mixer at 10 rpm for 4 minutes to attach DNA to the beads. When working with $\geq 5 \times 10^6$ cells, double the inversion time to 8 minutes; this is especially important if low agitation speeds were used during lysis. 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. 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 tube opening.



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. It is not necessary to remove all of the buffer at this point.

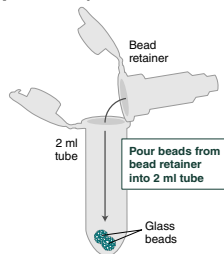


6. Place a labeled bead retainer into a Monarch Collection Tube II. Pour the beads into the bead retainer and close the cap. Discard the used Monarch 2 ml Tube. If working with multiple samples, be sure to close the cap after each bead transfer.

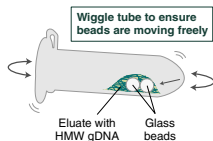


7. Pulse spin (≤ 1 second) the sample in a benchtop minicentrifuge to remove residual wash buffer from the beads.
8. Separate the bead retainer from the collection tube, pour the beads into a new, labeled Monarch 2 ml Tube, and insert the used bead

retainer into a labeled 1.5 ml microfuge tube (DNA low bind recommended, not provided). Discard the used collection tube.



9. Add 100 μ l 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 tube 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.

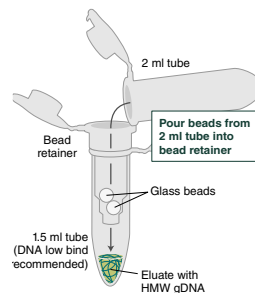


Questions? Our tech support scientists would be happy to help. Email us at info@neb.com

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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 (1 minute if working with $\geq 5 \times 10^6$ cells) at 12,000 $\times g$ 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 future use. The elution buffer (10 mM Tris, pH 9.0, 0.5 mM EDTA) is formulated for long term storage of gDNA.

