

Field Protocol for uHMW-DNA

August 2025



Materials

Plastics

○ 1 ml syringe and 21-gauge needle	Ensure needle is of sufficient length if performing cardiac puncture. For larger specimens, 5ml syringes can be used.
○ Wide-bore and standard bore pipette tips.	Use standard tips for reagents and wide-bore tips for samples, especially post-lysis.
○ Blood Storage Tube	Choice of tube depends on preservation method (e.g., flash-frozen, frozen, or ethanol-preserved); we typically use 2 mL tubes for ethanol. Label with SampleID-BS.
○ Blood Dilution Tube	1.5 mL Protein (or DNA) LoBind tube, labeled with SampleID-BD.
○ DNA Extraction Tube	1.5 mL Protein (or DNA) LoBind tube containing 40 µL of Proteinase K (20 ug/mL) and labeled with SampleID-DT.
○ DNA Storage and QC Tubes	1.5/2 mL DNA/Protein LoBind tubes permanently labeled with SampleID-uHMW. Aliquote for QC in SampleID-QC (quality control).
○ Forceps	To collect Nanobind disc during the extraction.

Reagents

○ 0.5M EDTA solution	Used to coat syringe and needle and impede clotting
○ Nanobind CBB kit	
○ Chilled ethanol >99% (optional)	Used for ethanol preservation of remaining blood for subsequent HMW-DNA extraction.
○ Isopropanol	Non-supplied reagent necessary for Nanobind extraction
○ 1X Phosphate Buffered Saline (PBS)	Non-supplied reagent necessary for Nanobind extraction used for diluting blood prior to extraction

Equipment

○ P200 and P1000 pipettes	  *Left:* Criemaq C-322 rotator (used in all homogenization steps). *Right:* 3D-printed magnetic rack. Project available here
○ Tube rotator, carousel type (Figure, leftside)	
○ Magnetic Rack (Figure, rightside)	



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Blood Drawing Procedure

1. Pull in and eject 0.5M EDTA using the needle and syringe
   Residual EDTA within the needle lumen and syringe hub is sufficient to impede clotting
2. Draw between 0.1 – 0.5ml blood by cardiac puncture and transfer to **Blood Storage Tube**
   Alternatively, blood can be drawn from the caudal vein.

When properly collected, blood remains viable for several days. We typically process batches of five specimens before proceeding with DNA extraction within 48 hours.



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DNA Extraction Protocol

1. Transfer 15ul blood to Blood Dilution Tube (with wide-bore tip)

The remaining blood can be flash-frozen in cryogenic temperatures for subsequent uHMW-DNA extraction or frozen at -20oC with or without ethanol for HMW-DNA extraction

2. Add 185ul 1X PBS with wide-bore tip and mix well by gentle pipetting
3. Transfer 200ul of diluted blood to Extraction Tube containing proteinase K.
4. Add 200 µL of Buffer BL3 to the sample followed by 20 µL of Buffer CLE3

Mix well (15x) with wide-bore tip and P1000 pipette set to 450ul to avoid bubbles

5. Rotate mixture in homogenizer for 30 min. at slow rotation (e.g. 9RPM)
6. Add Nanobind disc to Extraction Tube using a clean forceps

Whip tube to remove liquid from lid before opening to add Nanobind disc

7. Add 350ul isopropanol and inversion mix
8. Mix in rotator for 30min (R.T.)

At this point, large DNA fibers can be seen stuck to the disc if the procedure is successful

9. Bring down any liquid from the lid with a quick flick or whipping motion, then place the tube on the magnetic rack.

Additionally, remove any liquid from the lid using a standard pipette tip after opening the tube.

10. Discard liquid using a P1000 with standard pipette tips
11. Add 700ul wash buffer 1 (CW1), remove from magnet and inversion mix 4x

To minimize mechanical stress and prevent pellet dislodgement prior to inversion mixing, dispense solutions onto the wall opposite the pellet in this and subsequent steps.

12. Repeat 9 – 10
13. Add 500ul wash buffer 2 (CW2), remove from magnet and inversion mix 4x
14. Repeat 9 – 10
15. Add 500ul wash buffer CW2
16. Repeat 12 – 13

Remove as much liquid as possible to increase purity

17. Elute in 750 ultrapure water or Elution Buffer overnight at room temperature
18. Transfer 700–720 µL of eluate to the **DNA Storage** and 30–50 µL to the **QC** tubes.

uHMW-DNA is viscous and may require shearing prior to quantification/quantitation. Store extracts at –20 °C until sequencing for quality control.