STANDARD OPERATING PROTOCOL · EnGeL-MOLB-DNA-COL

DNA extraction from soil microarthropods with spin columns (NZY Tissue gDNA Isolation Kit)

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Area	Molecular biology (MOLB)
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Licence	Text: CC BY 4.0 · Code: MIT
Main hazards	 Kit lysis and wash buffers may contain chaotropic salts: never mix with bleach [TO CONFIRM against the NZY SDS] Ethanol: flammable; Proteinase K: respiratory sensitiser

1 Purpose and scope

This protocol extracts genomic DNA from soil microarthropods preserved in 96% ethanol, using spin columns (NZY Tissue gDNA Isolation Kit). It is the manual alternative to the robot extraction (EnGeL-MOLB-DNA-MAG), for smaller batches. Specimens are homogenised, so the method is destructive: specimens cannot be archived afterwards. Two homogenisation options are given: manual pestle grinding and mechanical bead beating.

2 Principle

Ethanol is removed by centrifugation, pipetting and evaporation. Specimens are homogenised and digested with Proteinase K in Buffer NT1. After lysis, Buffer NL and ethanol adjust the conditions so that DNA binds to the silica membrane of a spin column. Two wash buffers remove contaminants, the membrane is dried, and DNA is eluted in preheated Buffer NE (NZYtech, NZY Tissue gDNA Isolation Kit).

3 Before you start

Item	Details
Hands-on time	[TO CONFIRM]
Total time	[TO CONFIRM]. Fixed times: ethanol evaporation 15 min per concentrator load; lysis 1 h 30 min.
Sample amount	[TO CONFIRM: maximum amount per column]
Sample condition	Microarthropods in 96% ethanol at room temperature; ideally in the fridge if there is space
Prepare in advance	Ethanol added to Buffer NW2; ThermoMixer at 56 °C; Buffer NE at 70 °C; bench cleaned and UV on
Key safety points	See Safety and waste

4 Materials

4.1 Equipment

- IR vacuum concentrator: N-Biotek Micro-CenVac NB-503CIR with rotor NB-503-1524 (24 × 1.5 mL tubes)
- ThermoMixer or equivalent heated shaker (56 °C, 400 rpm)
- Microcentrifuge (≥ 14 000 × g)
- Bead-beating homogeniser [TO CONFIRM: model and settings] or pestles for manual homogenisation
- Vortex mixer
- Heat block at 70 °C (elution buffer)
- Spectrophotometer (e.g. NanoDrop) and fluorometer (e.g. Qubit) — see EnGeL-MOLB-NANODROP and EnGeL-MOLB-QUBIT

4.2 Consumables

- 1.5 mL and 2 mL microcentrifuge tubes
- NZYSpin Tissue columns and 2 mL collection tubes (in the kit)
- Corundum sand and ceramic beads (bead homogenisation)
- Filter tips; wide-bore tips (or regular tips with the end cut off) for ethanol removal

4.3 Reagents and kits

- NZY Tissue gDNA Isolation Kit (NZYtech, Cat. No. MB135 [TO CONFIRM: MB13502 or MB13503]), containing:
  - Buffer NT1
  - Proteinase K
  - Buffer NL
  - Buffer NW1
  - Buffer NW2 (add ethanol before first use)
  - Buffer NE
- RNase A (optional) [TO CONFIRM: supplier and concentration]
- Ethanol, 96–100% (binding step; NW2 preparation)

5 Reagent preparation

- **Buffer NW2:** add ethanol before first use, as stated on the bottle [TO CONFIRM: volume for the kit size].
- **Buffer NE:** preheat to 70 °C before elution.

6 Procedure

6.1 Before starting

- Clean the working space and material with bleach and water and leave the UV light on for at least 15 min.

- Use filter tips at all steps.
- Clean forceps and spatulas with 3% sodium hypochlorite and 96% ethanol between samples.
- Include extraction blanks (no specimens) processed exactly like the samples [TO CONFIRM: number per batch].
- Record each sample's EnGeL label in the lab book and the Lab Pipeline workbook.

6.2 Ethanol removal

CRITICAL Microarthropods are stored in 96% ethanol. Complete removal of ethanol is crucial before proceeding with DNA extraction, to avoid inhibition of downstream steps.

1. Centrifuge the microcentrifuge tubes at 6 000 × g for 3 min.
2. Carefully pipette out the supernatant ethanol without disturbing the pellet containing the microarthropods. It might be beneficial to use wide-bore tips or cut off the tip when using regular pipette tips.
3. Place the open tubes in the IR vacuum concentrator (N-Biotek Micro-CenVac NB-503CIR, rotor NB-503-1524; 5 000 rpm, 15 min, temperature [TO CONFIRM]) to evaporate residual ethanol. Monitor to ensure complete ethanol removal, but do not over-dry the sample.

6.3 Homogenisation and lysis

4. Add 180 µL of Buffer NT1 to each tube containing the dried microarthropods.
5. Homogenise the specimens [TO CONFIRM: steps for both options]: (a) manual grinding with a pestle in the tube; or (b) bead beating with corundum sand and ceramic beads [TO CONFIRM: amounts, instrument, speed and time].
6. Add 25 µL of Proteinase K to each tube.
7. Incubate in a ThermoMixer at 56 °C with 400 rpm for 1 h 30 min.
8. Optional (RNA removal): add 10 µL of RNase A, mix gently and incubate at room temperature for 5 min.

6.4 DNA purification

9. Add 200 µL of Buffer NL to each sample. Vortex for 10 s.
10. Add 210 µL of 96–100% ethanol. Vortex immediately.
11. Transfer the mixture to an NZYSpin Tissue column placed in a 2 mL collection tube. Centrifuge at > 11 000 × g for 1 min. Discard the flow-through and place the column in a new collection tube.
12. First wash: add 500 µL of Buffer NW1. Centrifuge at > 11 000 × g for 1 min. Discard the flow-through.
13. Second wash: add 600 µL of Buffer NW2 (ethanol added). Centrifuge at > 11 000 × g for 1 min. Discard the flow-through.
14. Optional, for samples with high inhibitor content: repeat the wash with another 600 µL of Buffer NW2.
15. Dry the membrane: place the column back in the collection tube and centrifuge at > 14 000 × g for 3 min.
16. Transfer the column to a clean 1.5 mL tube. Add 40 µL of Buffer NE (preheated to 70 °C) to the centre of the membrane.
17. Incubate at room temperature for 1 min, then centrifuge at 11 000 × g for 2 min to elute the DNA.

6.5 DNA storage and quality assessment

18. Store the eluted DNA at 4 °C for short-term use or at −20 °C for long-term storage.

PAUSE POINT Eluted DNA can be stored at 4 °C (short term) or −20 °C (long term) before quality assessment and downstream use.

19. Assess DNA purity by spectrophotometry (e.g. NanoDrop; A_{260}/A_{280} and A_{260}/A_{230} ratios) and quantify DNA concentration by fluorometry (e.g. Qubit), following EnGeL-MOLB-NANODROP and EnGeL-MOLB-QUBIT (or EnGeL-MOLB-PICOGREEN for plates). Record values in the database and the lab book.

6.6 After finishing

- Clean the bench with 70% ethanol.
- Empty the small bench bin into the right waste bin.
- Wash your hands.
- Turn off equipment and lights.
- Lock doors, fridge and freezer.

7 Calculations

Not applicable: volumes are per sample. For a batch of n samples, prepare $V = v \times n \times 1.1$ of each buffer (v: volume per sample, µL; 1.1: 10% excess).

8 Quality control

Control	Where	Purpose	Acceptance criterion
Extraction blanks	Each batch [TO CONFIRM: number]	Detect contamination	Processed through the PCR with the samples: no band on the gel, and < 5 ng/µL on the NanoDrop (regarded as noise)
Extraction positive control (mock community)	When establishing a new starting material or community	Check extraction performance	[TO CONFIRM: composition and criterion]
DNA purity	All extracts	A_{260}/A_{280}, A_{260}/A_{230} (EnGeL-MOLB-NANODROP)	Ideal: A_{260}/A_{280} 1.8–2.0 and A_{260}/A_{230} 2.0–2.2. Values outside are flagged, not failed: the PCR decides
DNA concentration	All extracts	Fluorometry (EnGeL-MOLB-QUBIT)	General rule: ≥ 10 ng/µL success; < 5 ng/µL fail; between 5 and 10 ng/µL the PCR result decides

The PCR is the best way to evaluate whether the DNA in a sample is viable.

9 Data and metadata

- **Sample labels:** EnGeL labels from the sample master data file; each DNA extract gets its own code (sample nature DNA), with the source sample code kept in a separate column.
- **Where:** the *Sample_Tracking* sheet of the Metabarcoding Lab Pipeline workbook and the lab book.

Minimum to record

- EnGeL sample label of the source sample and of the extract
- Extraction date, operator, homogenisation option used (pestle or bead beating)
- Kit lot number; RNase A lot (if used); deviations (extra NW2 wash, split sample)
- As in the Lab Pipeline *Sample_Tracking* sheet: Extraction_pipeline, Extraction_date, Equipment, DNA_ngul, A260/280, A260/230, Elution_vol_ul, DNA_total_ng

10 Safety and waste

Follow the manufacturer's instructions and safety data sheets for each reagent. Biological samples are potentially hazardous and are handled following the facility's procedures. EnGeL lab rules:

- Before you enter: wear your lab coat, use appropriate gloves, closed shoes only, tie back long hair.
- Not allowed: eating or drinking; touching phones or laptops with contaminated gloves; bags or coats on benches.
- Label all reagents (name, concentration, date) and treat all chemicals as hazardous.

Kit hazards (to be confirmed by the laboratory against the NZYtech SDS):

- Buffers containing guanidine salts: do not add bleach or acidic solutions to guanidine-containing waste, as highly reactive compounds can form. Keep this waste separate from the bleach used for bench and tool cleaning.

- Proteinase K may cause allergy or asthma symptoms if inhaled.
- Ethanol and isopropanol are flammable (to be confirmed against the SDS). Keep away from flames and heat sources.

Waste

EnGeL waste streams (choose the right bin):

- **Biological solid waste** (black bin, white bag): contaminated gloves, contaminated disposable materials, solid biological laboratory waste, contaminated PPE. No liquids or sharps.
- **Specific hazardous waste** (red bin, red bag): chemicals and pharmaceuticals designated for this waste stream, specific hazardous laboratory waste, anatomical material; sharps only in a rigid sharps container. No loose sharps.
- **Biological liquid waste** (jerrican, UN 3291): liquid biological waste and contaminated biological liquids only. Use a funnel; rinse the original container and the funnel with dH_2O and discard the rinse water in the same jerrican.

For this protocol: flow-through from the columns goes to biological liquid waste (jerrican), never with bleach; used columns, tubes and tips go to biological solid waste (black bin); unused or expired kit reagents go to the red bin.

11 Troubleshooting

Problem	Likely cause	Solution
Specimens aspirated with the ethanol	Narrow tip opening	Use wide-bore tips or cut off the tip of regular tips.
PCR inhibition	Residual ethanol, or inhibitors in the sample	Ensure complete ethanol removal; use the optional extra NW2 wash.
Low DNA yield	Incomplete homogenisation or lysis	[TO CONFIRM]
Column blocked	Too much material	[TO CONFIRM: maximum input; split the sample]

12 Remarks

The source protocol dried the specimens in a ThermoMixer (56 °C, 400 rpm, up to 1 h) after centrifuging at 10 000 × g; this version uses the ethanol removal of EnGeL-MOLB-DNA-MAG (decided 2026-11-06). The NZYtech insect support protocol homogenises up to 50 mg of insects under liquid nitrogen; this is an option to consider for the homogenisation step [TO CONFIRM].

13 References

NZYtech (2019). NZY Tissue gDNA Isolation kit – Support protocol for isolating genomic DNA from insects (MB135, v1901).

NZYtech (n.d.). NZY Tissue gDNA Isolation kit – manual [TO CONFIRM: version].

14 Version history and citation

Version	Date	Change	By
1.0.0	2026-11-16	First EnGeL-UC version, from the lab protocol “DNA Extraction Protocol for Soil Microarthropods” (NZY Tis-	Luís Cunha

Version	Date	Change	By
		sue gDNA Isolation Kit, 2026-07). Ethanol removal aligned with EnGeL-MOLB-DNA-MAG (decided 2026-11-06).	
1.0.1	2026-11-17	Contributors: Luísa Fraga and Andreia Quaresma.	Luís Cunha
1.0.2	2026-11-17	Authorship: all who contributed are authors, with their roles; lead author first where identified, reviewer (Luís Cunha) last.	Luís Cunha
1.1.0	2026-11-17	Storage of microarthropods in ethanol: room temperature, ideally in the fridge if there is space.	Luís Cunha
1.2.0	2026-11-20	Luísa Fraga confirmed as lead author; acceptance criteria for extraction blanks and DNA purity confirmed by Luís Cunha (as in EnGeL-MOLB-DNA-MAG).	Luís Cunha
1.2.1	2026-11-20	GHS pictograms for ethanol (harmonised EU classification); kit buffers still to be checked against their safety data sheets.	Luís Cunha
1.2.2	2026-11-20	Cross-references to the nucleic acid QC protocols, now split into EnGeL-MOLB-NANODROP, EnGeL-MOLB-QUBIT and EnGeL-MOLB-PICOGREEN.	Luís Cunha

How to cite

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15 Open questions

1. ORCIDs; DOI.
2. Homogenisation steps: pestle (when, how long) and bead beating (instrument, amount of corundum sand and beads, speed, time). Liquid nitrogen as in the NZYtech insect protocol?
3. Kit catalogue number (MB13502 or MB13503); ethanol volume for Buffer NW2; RNase A supplier and concentration.
4. Number of extraction blanks per batch; positive control composition.
5. Hands-on and total time; maximum sample amount per column.
6. Troubleshooting for low yield and blocked columns.