# Bench sheet · EnGeL-MOLB-DNA-COL

**DNA extraction from soil microarthropods with spin columns (NZY Tissue gDNA Isolation Kit)** · version 1.2.2 (2026-11-20). Full protocol: EnGeL-MOLB-DNA-COL. Tick each step when done; write deviations in Notes.

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|---|---|
| Date | |
| Operator | |
| Plate ID / samples | |
| NZY Tissue gDNA Isolation Kit lot | |
| RNase A lot (if used) | |

## Before starting

| ☐ | No. | Step |
|---|---|---|
| ☐ | • | 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. |

## Ethanol removal

| ☐ | No. | Step |
|---|---|---|
| ! | | **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. |

## Homogenisation and lysis

| ☐ | No. | Step |
|---|---|---|
| ☐ | 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. |

## DNA purification

| ☐ | No. | Step |
|---|---|---|
| ☐ | 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. |

## DNA storage and quality assessment

| ☐ | No. | Step |
|---|---|---|
| ☐ | 18 | Store the eluted DNA at 4 °C for short-term use or at −20 °C for long-term storage. |
| ☐ | 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. |

## After finishing

| ☐ | No. | Step |
|---|---|---|
| ☐ | • | 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. |

**Notes / deviations**

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