Bench sheet · EnGeL-MOLB-DNA-MAG

DNA extraction from bulk soil fauna samples with magnetic beads (Omega Bio-tek Mag-Bind® Blood & Tissue DNA HDQ 96, Invitek InviGenius®) · version 1.3.2 (2026-11-20). Full protocol: EnGeL-MOLB-DNA-MAG. Tick each step when done; write deviations in Notes.

Date	
Operator	
Plate ID / samples	
Mag-Bind® Blood & Tissue DNA HDQ 96 Kit (M6399) lot	
RNase A lot (if used)	
100% ethanol / isopropanol lots used for buffer preparation	

Binding mix (prepare fresh for each run). Prepare a master mix of HDQ Binding Buffer and Mag-Bind® Particles HDQ only for the samples to be extracted in this run (the mix does not keep). Resuspend the Mag-Bind® Particles thoroughly before pipetting. Volumes include 10% excess (see Calculations).

Component	Per sample	24 samples (+10%)	48 samples (+10%)	96 (92 samples + 4 blanks, +10%)
HDQ Binding Buffer	320 µL	8 448 µL	16 896 µL	33 792 µL
Mag-Bind® Particles HDQ	20 µL	528 µL	1 056 µL	2 112 µL
Total binding mix	340 µL	8 976 µL	17 952 µL	35 904 µL

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.
☐	•	Set the dry bath to 55 °C.
☐	•	Check that the kit buffers have been prepared (Reagent preparation).
☐	•	Samples are processed in 96-well format: each run comprises 92 samples and 4 extraction blanks (negative controls) at positions H3, H6, H9 and H12, processed exactly like the samples.
☐	•	Prepare the plate map (sample sheet) before starting and record it in the database and the lab book (Annex 1).
☐	•	When establishing a new starting material or community, include an extraction positive control (e.g. a mock community of known composition) and record its well in the plate map [TO CONFIRM: composition].

Pre-treatment (choose by sample type)

A. Microarthropods in ethanol: 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.

B. Nematode suspensions in water: water removal

☐	No.	Step
☐	4	Thaw the frozen tubes containing the nematode suspension [TO CONFIRM: thawing conditions].
☐	5	Centrifuge the tubes at 6 000 × g for 3 min to pellet the nematodes.
☐	6	Carefully remove the supernatant with a pipette without disturbing the nematode pellet, leaving 50 µL of water with the nematode pellet. It might be beneficial to use fine tips and to work against a dark background to check the pellet.

C. Macroarthropods: drying and powdering

[TO CONFIRM: drying conditions, powdering method, and amount of powder per extraction.]

Lysis

☐	No.	Step
☐	7	Add 250 µL of TL Buffer to each tube.
☐	8	Add 20 µL of Proteinase K to each tube. Vortex at 1 500 rpm to mix.

☐	No.	Step
☐	9	Incubate the tubes in a dry bath at 55 °C for 1 h 30 min [TO CONFIRM: 1 h 30 min in the UC v2.0 protocols; “usually 2 h” stated on 2026-11-16], vortexing at 1 500 rpm every 30 min.
!		**CRITICAL** For non-destructive lysis of microarthropods, do not extend the incubation, so that specimens remain intact for archiving. Shorter times give less but higher-quality DNA; longer times give more but degraded DNA, particularly for soil animals.
☐	10	Optional: add 5 µL of RNase A, vortex at 1 500 rpm to mix and leave at room temperature for 2 min.
☐	11	Centrifuge the tubes at 10 000 × g for 5 min to settle the specimens and debris.
☐	12	Transfer 200 µL of the lysate (supernatant) to a 96 deep-well plate (Bind plate), following the plate map, without aspirating specimens.
☐	13	Optional but desired (microarthropods): recover the specimens for archiving. Remove the remaining lysate, refill the tube with 96% ethanol and place a unique, laser-printed coded label inside the tube. Store the tubes in 96-position cryoboxes and archive them in the collection.

Automated DNA purification (InviGenius®)

☐	No.	Step
☐	14	Add 230 µL of AL Buffer to each well of the Bind plate. Seal the plate and mix on a plate vortex/shaker at 1 500 rpm for 10 min.
☐	15	In the meantime, prepare three deep-well wash plates and one deep-well elution plate: · W1 (VHB1 plate): 500 µL VHB Buffer per well · W2 (VHB2 plate): 500 µL VHB Buffer per well · W3 (SPM plate): 500 µL SPM Buffer per well · Elution plate: 50 µL Elution Buffer per well (single elution)
☐	16	Prepare the binding mix (HDQ Binding Buffer + Mag-Bind® Particles HDQ) only for the samples to be extracted in this run (Reagent preparation).
!		**CRITICAL** Resuspend the Mag-Bind® Particles thoroughly before pipetting. The binding mix does not keep: prepare it only for the samples of each run.
☐	17	DNA binding: add 340 µL of binding mix to each well of the Bind plate. Mix thoroughly by pipetting up and down 10 times.
☐	18	Load the robot according to the following plate positions:

Plate	Position
Tip plate	1
Bind plate (lysate + AL Buffer + binding mix)	2
VHB1 plate	3
VHB2 plate	4
SPM plate	5
Elution plate	8

☐	No.	Step
!		**CRITICAL** Before starting the InviGenius® run, check that all plates are in the correct robot positions.
☐	19	Run the protocol “M6399_IG96_v1” (c. 45 min): binding with 10 min mixing, two VHB washes and one SPM wash (2 min each), 10 min bead drying, and elution for 7 min at 65 °C. Full program parameters are given in Annex 2.
☐	20	Unload the plates. Transfer the eluate (purified DNA) to a labelled 96-well microplate with a red frame.
☐	21	Discard the Bind (lysate) plate and the deep-well elution plate in the biological hazard container. Send the wash plates to be washed and autoclaved.

DNA storage and quality assessment

☐	No.	Step
☐	22	Store the eluted DNA at 4 °C for short-term use or at −20 °C for long-term storage.
☐	23	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
☐	•	Empty the liquid left in the wash plates into the biological liquid waste before sending the plates to be washed (see Safety and waste).
☐	•	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
