Bench sheet · EnGeL-MOLB-RNA

Total RNA extraction with TRIzol and spin-column clean-up (honey bees; isopod hepatopancreas) · version 1.3.0 (2026-11-20). Full protocol: EnGeL-MOLB-RNA. Tick each step when done; write deviations in Notes.

Date	
Operator	
Plate ID / samples	
TRIzol lot	
RNA column kit lot	

Sample-type settings.

Step	A. Bees	B. Isopod hepatopancreas
TRIzol	300–500 µL	300 µL
Homogenisation	Bead beating 6.5 m/s, 2 × 30 s	Mechanical homogeniser; then 5 min at room temperature
Chloroform	200 µL, mix by pipetting	150 µL, vortex
Phase separation	14 000 × g, 15 min, 4 °C	15 000 × g, 15 min, 4 °C
Cold 70% ethanol	350 µL	250 µL
Column kit	NZY Total RNA (NI, DNase, NWR1, NWR2)	RNeasy-type (RW1, RPE)

Before starting

☐	No.	Step
☐	•	Set the centrifuge to 4 °C.
☐	•	Clean the bench and work with RNase-free tubes and filter tips [TO CONFIRM].

A. Honey bees

☐	No.	Step
☐	1	Put 300–500 µL of TRIzol and the bee in a tube with beads.
☐	2	Homogenise in the bead-beating machine at 6.5 m/s for 30 s, twice.
☐	3	Centrifuge at 4 000 × g for 3 min at 4 °C.
☐	4	Transfer the liquid to a new tube for the chloroform step [TO CONFIRM: volume transferred].
☐	5	Add 200 µL of chloroform and mix well by pipetting.
☐	6	Incubate for 3 min at room temperature.
☐	7	Centrifuge at 14 000 × g for 15 min at 4 °C. Three phases form (pink at the bottom, a white layer in the middle and a colourless supernatant on top); take as much of the upper phase as possible.
☐	8	Add the upper phase to 350 µL of cold 70% ethanol and pipette up and down.
☐	9	Change gloves.
☐	10	Transfer to the (blue) column and centrifuge at 11 000 × g for 30 s. If needed, load the sample in portions of 500 µL. Discard the flow-through.
☐	11	Add 350 µL of Buffer NI and centrifuge at 11 000 × g for 30 s. Discard the flow-through.
☐	12	Prepare the DNase digestion mix (90 µL DI + 10 µL DNase I per sample). Add 95 µL to the column and incubate for 15 min at room temperature.
☐	13	Add 200 µL of Buffer NWR1, centrifuge at 11 000 × g for 1 min and discard the flow-through.
☐	14	Add 600 µL of Buffer NWR2, centrifuge at 11 000 × g for 1 min and discard the flow-through.
☐	15	Add 250 µL of Buffer NWR2, centrifuge at 11 000 × g for 1 min and discard the flow-through.
☐	16	Place the column in a new microtube and centrifuge at 11 000 × g for 2 min to remove residual buffer.
☐	17	Place the column in a new microtube, add 20 µL of RNase-free water to the column and wait 1 min.
☐	18	Change gloves. Centrifuge at 11 000 × g for 1 min: the eluate is sample 1.
☐	19	Place the column in a new microtube, add 20 µL of RNase-free water and wait 1 min. Centrifuge at 11 000 × g for 1 min: the eluate is sample 2.
☐	20	Measure concentration and quality on the NanoDrop (EnGeL-MOLB-NANODROP).

B. Isopod hepatopancreas

☐	No.	Step
☐	21	Homogenise the hepatopancreas in 300 µL of TRIzol in a mechanical homogeniser.
☐	22	Incubate for 5 min at room temperature.
☐	23	Add 150 µL of chloroform and vortex well.

☐	No.	Step
☐	24	Incubate for 3 min at room temperature.
☐	25	Centrifuge at 15 000 × g for 15 min at 4 °C. Three phases form (pink at the bottom, a white layer in the middle and a colourless supernatant on top); take as much of the upper phase as possible.
☐	26	Add the upper phase to 250 µL of cold 70% ethanol and pipette up and down.
☐	27	Transfer to the column and centrifuge at 8 000 × g for 10 s. If needed, load in portions of 500 µL. Discard the flow-through.
☐	28	Add 700 µL of 100% ethanol and centrifuge at 8 000 × g for 15 s. Discard the flow-through. This extra wash is intended (lab practice); it is not part of the standard RNeasy procedure.
☐	29	Add 700 µL of Buffer RW1 and centrifuge at 8 000 × g for 15 s. Discard the flow-through.
☐	30	Add 500 µL of Buffer RPE and centrifuge at 8 000 × g for 15 s. Discard the flow-through.
☐	31	Add 500 µL of Buffer RPE and centrifuge at 8 000 × g for 3 min. Discard the flow-through.
☐	32	Place the column in a new microtube and centrifuge at ≥ 12 000 × g (microcentrifuge, 1.5 mL tube rotor) for 2 min to remove residual buffer.
☐	33	Place the column in a new microtube, add 20 µL of RNase-free water and wait 1 min. Centrifuge at 8 000 × g for 15 s: the eluate is sample 1.
☐	34	Place the column in a new microtube, add 20 µL of RNase-free water and wait 1 min. Centrifuge at 8 000 × g for 15 s: the eluate is sample 2.
☐	35	Measure concentration and quality on the NanoDrop (EnGeL-MOLB-NANODROP).

RNA storage

☐	No.	Step
☐	36	Store the RNA at [TO CONFIRM: −80 °C?] until use.

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
