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Version 1

DNeasy Blood & Tissue Kit for eDNA analysis of stomach contents of shrimps V.1

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Protocol status: Working

We use this protocol and it's working



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Abstract

DNA extraction for eDNA analysis of stomach contents of shrimps

Notes before starting

- * Perform all centrifugation steps at room temperature (15-25°C)
 - * Redissolve any precipitates in Buffer AL and Buffer ATL
 - * Buffer AL should be premixed with ethanol before use (Add 90ml of EtOH to bottle containing 86ml Buffer or 260 ml EtOH into a bottle containing 247 ml Buffer and shake thoroughly. Mark bottle.
 - * Add ethanol to Buffer AW1 and Buffer AW2 concentrates and mark on the bottle
 - * Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm up to 56°C for 5 min until the precipitates have fully dissolve
 - * Mix Buffer AW1 before use by inverting several times
 - * Equilibrate frozen tissue or cell pellets to room temperature
 - * Preheat an incubator to 56°C

Protocol

- Tissue:** For rodent tails, use 0.025 g (25 mg) of tissue.
Add 180 µl **Buffer ATL**.
Add 20 µl **proteinase K**, mix by vortexing and incubate at 56°C **overnight** (until completely lysed). Vortex/mix during incubation. Vortex 15 sec directly before proceeding to step 2.
- Add 200 µl **Buffer AL**. Immediately mix by vortexing.
Incubate sample at 56°C for 10 min.
- Add 200 µl **Ethanol** (100%). Mix thoroughly by vortexing.
- Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube.
Centrifuge at 20,000 x g for 1 min.
Discard the flow through and collection tube.
- Place the spin column in a new 2 ml collection tube.
Add 500 µl **Buffer AW1**. Centrifuge for 1 min at 20,000 x g.
Discard flow-through and collection tube.
- Place the spin column in a new 2 ml collection tube.
Add 500 µl **Buffer AW2**. Centrifuge for 3 min at 20,000 x g.
Discard flow-through and collection tube.
- Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.





- 9 Preheat **Buffer AE** at 56°C before adding in. 
- 10 Elute the DNA by adding 100 µl **Buffer AE** to the center of the spin column membrane.
Incubate for 1 min at room temperature (15-25°C).
Centrifuge for 1 min at 6000 x g.
- 11 Optional: Repeat step 8 for increased DNA yield, collect DNA in a separate microcentrifuge tube. 