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We use this protocol and it's working

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Inexpensive DNA Extraction Protocol

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ABSTRACT

This is a protocol for doing extraction of DNA using inexpensive reagents rather than enzymes or kits. It can be used for plant, animal, or fungus samples.

MATERIALS

Forceps (for handling sample)
Pestle (for grinding sample in microtube)
Hinged 1.5 µL microtubes (3 per sample)
Microcentrifuge
Water bath
Adjustable micropipette 1-10 µL
Adjustable micropipette 10-100 µL
Adjustable micropipette 100-1000 µL
Pipette tips
Molecular grade water (100 µL per sample)
Silica resin (silica dioxide 50% w/v in water) (3 µL per sample)
Wash buffer (diluted 1:1 in 95% ethanol) (1 mL per sample)
Guanidine Hydrochloride 6M (250 µL per sample)

PROTOCOL MATERIALS

Guanidine Hydrochloride 6M **Carolina Biological Supply Catalog #**C33427	Step 5
Silica Resin **Carolina Biological Supply Catalog #**C33426	Step 12
Wash Buffer **Carolina Biological Supply Catalog #**C33428	In 3 steps

Prepare sample and equipment

1 Make sure all instruments, such as forceps and pestle, are clean and sterile. Make sure Wash Buffer **Carolina Biological Supply Catalog #**C33428 has been diluted 1:1 in 95% ethanol.

2 Prepare water bath at 65 °C .

3 Dissect sample from specimen. (This will be a piece of tissue approximately 10–20 mg. For small arthropods such as beetles or spiders, 1 or 2 legs will typically suffice.) Return specimen to freezer.

4 If sample was stored in ethanol, let sample dry for 5–10 minutes.

5 Prepare a clean 1.5 mL hinged tube by writing sample ID on it and filling with 250 µL of Guanidine Hydrochloride 6M **Carolina Biological Supply Catalog #**C33427

Lyse cells

11m

6 Put sample in tube. Grind sample with pestle until broken up into tiny pieces.

7 Incubate sample tube in 65 °C water bath for 00:10:00 . 10m

8 Remove tube and lower temperature of water bath to 57 °C .

9 Centrifuge tube for 00:01:00 at maximum speed to pellet debris. 1m

10 Remove Silica Resin **Carolina Biological Supply Catalog #**C33426 from refrigerator.

11 Label a clean 1.5 µL tube with sample number. Transfer 150 µL of the supernatant to the clean tube. Discard old tube containing debris.

Bind DNA

5m 30s

12 Add 3 µL of Silica Resin **Carolina Biological Supply Catalog #**C33426 to tube. Mix well by pipetting up and down several times.

13 Close tube and incubate for 00:05:00 in 57 °C water bath. 5m

14 Centrifuge for 00:00:30 at maximum speed to pellet the resin. 30s

15 Use a pipette with a fresh tip to remove the supernatant, being careful not to disrupt the pellet.

Wash

1m

16 Remove molecular grade water from refrigerator and Wash Buffer **Carolina Biological Supply Catalog #**C33428 from freezer.

17 Add 500 µL of ice-cold Wash Buffer **Carolina Biological Supply Catalog #**C33428 to the pellet. Mix well by pipetting up and down several times to resuspend the silica resin.

18 Close the tube and centrifuge for 00:00:30 at maximum speed to pellet the resin. 30s

19 Use a pipette with a fresh tip to remove the supernatant, being careful not to disrupt the pellet.

20 Again, add 500 µL of ice-cold Wash Buffer **Carolina Biological Supply Catalog #**C33428 to the pellet. Mix well by pipetting up and down to resuspend the silica resin.

21 Close the tube and centrifuge for 00:00:30 at maximum speed to pellet the resin. 30s

22 Return wash buffer to freezer.

23 Use a pipette with a fresh tip to remove the supernatant, being careful not to disrupt the pellet. Spin the tube briefly to collect any remaining drops of supernatant, and then remove these with a pipette.

Elute DNA

30s

24 Add 100 µL of molecule grade water to the silica resin and mix by pipetting up and down several times.

25 Incubate the mixture at 57 °C for 5 minutes.

26 Centrifuge for 00:00:30 at maximum speed to pellet the resin.

30s

27 Label a clean 1.5 µL tube with sample number. Transfer 90 µL of the supernatant to the clean tube, being careful not to disturb the pellet. Discard old tube containing the resin.

28 Store sample in freezer until ready to PCR. If going directly to PCR, put sample in refrigerator.