DNAbsolute

Protocol

idylle

1. Before your start

Kit contents

- 3M DNAbsolute solution
- 150 mM PBS buffer
- 25 mM Tris buffer
- Lysis buffer 1
- Lysis buffer 2

Other materials you need

- 20 mg/mL proteinase K
- 96-100% ethanol
- 70% ethanol
- 10 mg/mL RNase A (optional)
- 1.5 mL DNAse-free tubes (1 per sample)
- Refrigerated centrifuge (10,000 g)

Storage

PBS, Tris & Lysis buffers are stable for 1 year at room temperature (15-25°C).

The 3M DNAbsolute reagent shoud be stored at 4°C and is stable for 1 year.

Important notes

- DNAbsolute is efficient at recovering DNA fragments >300 bp. It will work best with fresh material or material that has been stored immediately at ≤ -20°C with limited thawing & freezing cycles. Poor quality, and/or fragmented starting material will result in reduced DNA yield.

- All centrifugation steps are carried out at 10,000 g. When working from small amounts of starting material, increasing the centrifugation speed up to 13,200 g may help making the DNA pellet more visible and minimize the risk of disturbing or loosing it when collecting the supernatant.

- A white precipitate may form in Lysis buffers 1 & 2 upon storage. If necessary, warm at 56°C until the precipitates have fully dissolve before starting the lysis protocol.

- The lysis protocol described in Step 2 has been optimized for insect DNA extraction. Depending on your sample type, your lysis method of choice can be used instead before proceeding to Step 3. Please contact us if you need any advice.

- Volumes of solutions used in **Steps 2 & 3** need to be adjusted according to the weight of your starting material. Please refer to **Table 1** below for recommended volumes.

Weight of starting material	Up to 10 mg (i.e. single specimens)	Up to 50 mg	Up to 100 mg
Lysis buffer 1	36 µL	180 µL	360 µL
Proteinase K	4 µL	20 µL	40 µL
Lysis buffer 2	40 µL	200 µL	400 µL
96-100% ethanol	40 µL	200 µL	400 µL
DNAbsolute	20 µL	100 µL	200 µL

Table 1. Recommended volumes to use in Steps 2 & 3 depending on starting material weight

2. Sample lysis

• Place up to 100 mg of sample in a 1.5 mL DNase-free tube.

• Add lysis buffer 1 to your sample according to volumes described in Table 1.

For a non-destructive lysis, skip the following step and proceed immediately to proteinase K addition.

• (Optional) Grind the sample using a pestle.

• Add proteinase K.

• Mix thoroughly by vortexing and incubate at 56 °C until the sample is completely lysed.

To improve sample dispersion, vortex the tube occasionally, place it in a thermomixer, a shaking water bath or on a rocking platform during lysis.

The lysis is usually completed within 1 hour. Depending on your specimen composition, and whether it was ground or kept intact, total incubation time may be extended up to 24h.

• Centrifuge 3 min at 10,000 g at 4° C and recover the supernatant.

• If RNA-free genomic DNA is required, add 4 µL of RNase A, mix by vortexing, and incubate for 2 min at room temperature (15–25°C) before proceeding to the next step.

• Add lysis buffer 2 according to volumes described in Table 1 and mix thoroughly by vortexing.

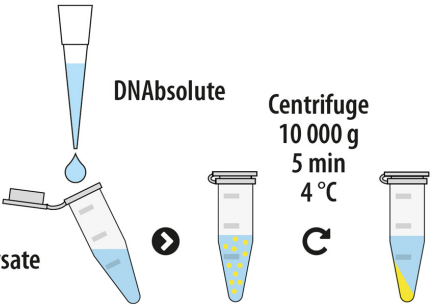
• Add 96-100% ethanol according to volumes described in Table 1 and mix again thoroughly by vortexing.

3. DNA precipitation

• Add 3M DNAbsolute reagent according to volumes described in Table 1 to the whole lysate volume.

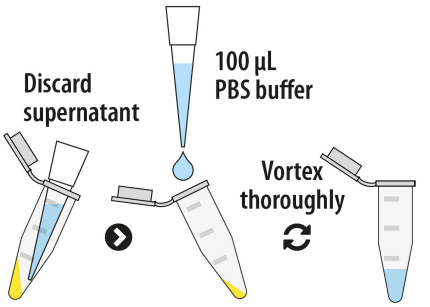
• Vortex thoroughly.

• Centrifuge 5 min at 10,000 g at 4°C.



• Discard the supernatant.

• Recover the pellet with 100 µL of PBS buffer and mix thoroughly by vortexing until the pellet is dissolved.



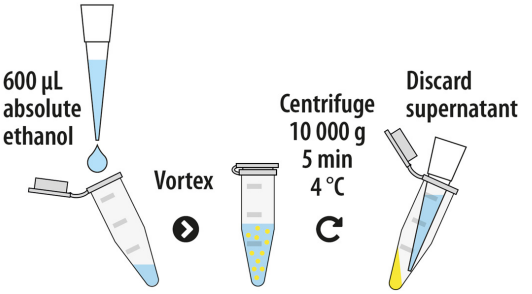
4. DNA purification

• Add 600 µL of 96-100% ethanol.

• Vortex thoroughly.

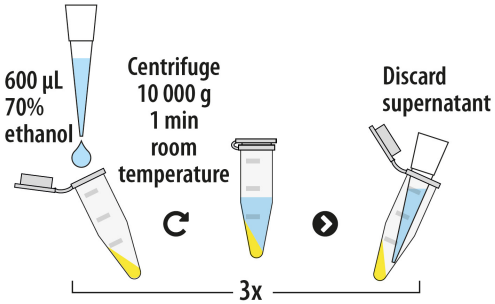
• Centrifuge 5 min at 10,000 g at 4°C.

• Discard the supernatant.



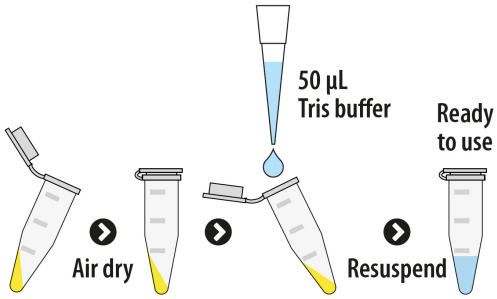
Wash the pellet 3 times:

- Add 600 µL of 70% ethanol.
- Centrifuge for 1 min at 10,000 g at room temperature.
- Discard the supernatant.



- Air dry the pellet.
- Resuspend the pellet in 50 µL of Tris buffer.

Resuspend in a smaller volume (i.e. 25 µL) for more concentrated DNA or when working with low sample amounts.



5. Quality control

- The obtained DNA yield & purity can be assessed using a spectrophotometer (A260/A280 ~1.8–2.0; A260/A230 ~2.0–2.2).
- You can also check the purity by running the DNA on an electrophoresis gel.

Check our website for example results, updated FAQ and much more

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