A&A BIOTECHNOLOGY
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Manual

Genomic Midi AX Plant

Increased efficiency kit for genomic DNA purification from plant tissues.
Procedure with DNA precipitation.

catalog #	size
050-20M	20 isolations

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Table of Contents

Contents

component	050-20M	storage
Spin 100AX columns	20 pcs	2–8 °C
15 ml tubes	40 pcs	15–25 °C
Counterweight column	1 pcs	15–25 °C
LS lysis suspension	110 ml	15–25 °C
K2 wash solution	100 ml	15–25 °C
K3 elution solution	25 ml	15–25 °C
TE buffer	100 ml	15–25 °C
Isopropanol	20 ml	15–25 °C
Proteinase K	2 x 1.1 ml	2–8 °C

The binding capacity of the DNA purification column is 100 µg of DNA.

Additional equipment and reagents

Necessary

- 2 ml sterile Eppendorf tubes
- 15 ml Falcon tubes
- 70% ethanol
- Incubator or thermoblock set to 50 °C
- Vortex
- Centrifuge with swing-out rotor for 15 ml tubes
- Microcentrifuge

Optional

- RNAse (cat. # 1006-10, 1006-50)
- Sterile water (cat. # 003-075, 003-25)

Isolation protocol

1. Transfer up to **200 mg** of **dried, powdered plant material** or up to **1 g fresh / frozen blood cut plant tissue** to a 2 ml Eppendorf tube (not included).

2. LS lysis suspension should be mixed by inverting the tubes before use.

   Add **5 ml** of **LS** lysis suspension and **100 µl** of **proteinase K**.

3. Vortex the sample and incubate for **30 min** at **50 °C**. Mix the samples by inverting the tubes a few times.

   The incubation step can be performed in Eppendorf Thermomixer or analogous equipment at 1400 RPM and 50 °C.

   RNA digestion (optional): add 10 µl of RNAse (10 mg/ml) (not included). Mix and incubate for 5 min at room temp.

4. Vortex the sample for **15 s**.
   Centrifuge for **5 min** at **10 000 RPM**.

   The DNA pellet should be visible at the bottom of the tube. It is a mixture of non-lysed fragments of sample material and particles of the LS lysis suspension.

5. Apply the supernatant onto the Spin 100AX column placed inside a 15 ml tube.

   Note: If you have an odd number of samples, please remember about counterweight columns before centrifugation.

   Centrifuge in a swing-out rotor for **2 min** at **3000 RPM** (1500 x g).

6. Transfer the Spin 100AX column to **a new** 15 ml tube (included).

7. Add **2.5 ml** of **K2** wash solution. Centrifuge in a swing-out rotor for **2 min** at **3000 RPM** (1500 x g).

8. Add again **2.5 ml** of **K2** wash solution. Centrifuge in a swing-out rotor for **2 min** at **3000 RPM** (1500 x g).

9. Transfer the Spin 100AX column to **a new** 15 ml tube (included).

10. Add **550 µl** of **K3** elution solution. Keep for **2 min** at **room temp.**

11. Centrifuge in a swing-out rotor for **1 min** at **3000 RPM** (1500 x g).

12. Add again **550 µl** of **K3** elution solution.
    Centrifuge in a swing-out rotor for **1 min** at **3000 RPM** (1500 x g). Remove the Spin 100AX column.

13. Transfer the eluate to **a new** 2 ml tube (not included).

14. Add **800 µl** of **isopropanol**. Close the tube with a cap and mix carefully by inverting the tube a few times.

If white precipitate is present in the tubes, follow point A.
If white precipitate is not present in the tubes, follow point B.

A. The white precipitate is visible

1. Centrifuge for **2 min** przy **4000 RPM**. Carefully discard supernatant.
2. Add **500 µl** of **70% ethanol** (not included).
3. Centrifuge for **2 min** przy **4000 RPM**. Carefully discard supernatant.
4. Air dry the DNA pellet for **10 min** at **room temp.** up-site-down.
5. Dissolve the DNA pellet in the desired volume of **TE** buffer (included) or sterile nuclease-free water (not included).

   To dissolve the DNA easily and completely the sample can be incubated at 50 °C and gently mixed occasionally by subtle shaking.
6. Store the DNA at 4-8 °C or -20 °C until later use.

B. The white precipitate is not visible

1. Transfer the samples to new centrifuge tubes suitable for high centrifuge speed.
   Centrifuge for **15 min** przy **12 000- 14 000 RPM**. Carefully discard supernatant.
2. Add **500 µl** of **70% ethanol** (not included).
3. Centrifuge for **5 min** przy **12 000- 14 000 RPM**. Carefully discard supernatant.
4. Air dry the DNA pellet for **10 min** at **room temp.** up-site-down.
5. Dissolve the DNA pellet in the desired volume of **TE** buffer (included) or sterile nuclease-free water (not included).

   To dissolve the DNA easily and completely the sample can be incubated at 50 °C and gently mixed occasionally by subtle shaking.
6. Store the DNA at 4-8 °C or -20 °C until later use.

Safety information

DANGER

Proteinase K

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor/physician.

WARNING

LS lysis suspension

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

K2 wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

K3 elution solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

Isopropanol

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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