Quick Start

GenElute™-E Single Spin Plant DNA 96 Kit

For 96-Well Purification of Genomic DNA from Plant Samples

EC596

Quick-Start Protocol (See Standard Protocol for detailed instructions.)

Lysis

- Add 10 – 50 mg of plant tissue sample per well of Tube Strips.
- If using beads for homogenization, add them now to sample containing wells.
- Prepare Bead-Beating Master Mix, add 100 µL of the Bead-Beating Master Mix per well of Tube Strips.

Number of samples	1	96 (+20%)
Bead-Beating Buffer BB	99 µL	11,404.8 µL
RNase A Plant R	1 µL	115.2 µL
Final Volume	100 µL	11,520 µL

- Seal Tube Strips tightly with Cap Strips.
- Perform sample homogenization.
- Centrifuge 1 minute at 1,000 x g. Remove Cap Strips.
- Prepare Lysis Master Mix, add 105 µL of the Lysis Master Mix per well of Tube Strips.

Number of samples	1	96 (+20%)
Plant Lysis Buffer LB	100 µL	11,520 µL
SmartLyse™ P Protease P	5 µL	576 µL
Final Volume	105 µL	12,096 µL

- Seal with Cap Strips. Incubate 30 minutes at 60 °C, maximum agitation.
- Incubate 10 minutes at 80 °C, maximum agitation.
- Detach Cap Strips and add 25 µL Clearing Solution P CS. Mix by pipetting.
- Centrifuge Tube Strips for 3 minutes at maximum speed.

Preparation of Purification Plate

(during 60 °C and 80 °C incubation)

- Detach lower and upper sealing foils from Purification Plate.
- Place Purification Plate on top of a Conditioning Plate.
- Centrifuge 1 minute at 1,000 x g to collect void buffer.
- Place conditioned Purification Plate on top of Storage Plate.

Purification of DNA

- Transfer lysis supernatant from Tube Strips to Purification Plate.
- Centrifuge 1 minute at 1,000 x g to collect DNA into the Storage Plate.
- Collected DNA is ready to use.

Intended Use

For 96-well plate purification of genomic DNA from plant tissue samples. This protocol has been developed for 10–50 mg plant tissues like leaves, blossoms, fruits, roots, flour and seed samples. 30 mg is generically recommended (for certain plant species, optimization of input amount may be required).

Storage and Stability

Store SmartLyse™ P Protease (P), RNase A Plant (R) and Purification Plate at 2-8 °C. The remaining components should be stored at room temperature. Use the kit within 12 months of manufacture.

Kit Contents

- Tube Strips and Cap Strips: 8-well strips of tubes and compatible strip caps.
- Purification Plate: 96-well plate containing the resin matrix for DNA purification.
- DNA Storage Plate: 96-well plate for the collection of the purified DNA.
- Adhesive Foils for plate sealing after lysis.
- Reagents:
  - Plant Lysis Buffer (LB)
  - Bead-Beating Buffer (BB)
  - RNase A Plant (R)
  - SmartLyse™ P Protease (P)
  - Clearing Solution P (CS)
  - 1x Tris Buffer (T).

Materials and Equipment Needed

- Conditioning Plate: 96-deep well plate with minimum of 800 µL well volume for the collection of void volume during preparation of the Purification Plate. Reusable!
- 96-well swing-out centrifuges

  Important: Switch centrifuge to relative centrifugal force, rcf (x g); if this is not possible please use formula to calculate the conversion of round per minute (rpm) into rcf. Most centrifuges offer the choice between rpm and g-force (rcf); if not, calculate the rpm matching the g-force using the formula:

  $rpm = 1{,}000 \times \sqrt{(\ g/(1.12 \times r))}$,
  where r = radius of rotor in mm
  and g is the required g-force.
- 96-well Plate Thermal Shaker with agitation, capable of heating to 60 °C and 80 °C.

  Alternative: Heating Block or heat chamber.
- Vortex device.
- Pipets for 10 µL, 200 µL, and 1,000 µL scales, corresponding pipet tips.
- 8-channel pipets for 200 µL scale, corresponding pipet tips.
- Troughs for Master Mix preparation(s) holding >10 ml.
- Balance Plate(s) to be used in the centrifuge in case an odd number of plates is processed.
- If performing bead-beating for sample homogenization, a bead-beater device compatible with Tube Strips will be needed.

Preparation before starting

- Heat the thermal shaker or heating block/chamber to 60 °C.
- Set the centrifuge to 1,000 x g.

Standard Protocol

Lysis

1. Add 10–50 mg of plant tissue sample per well of Tube Strips.
2. If using beads for homogenization, add them now to sample containing wells.
3. For individual loading, transfer 99 µL Bead-Beating Buffer (BB) and 1 µL RNase A Plant (R) to each well. Otherwise, prepare Bead-Beating Master Mix with 20% excess volume for the number of samples (see table).

Number of samples	1	96 (+20%)
Bead-Beating Buffer (BB)	99 µL	11,404.8 µL
RNase A Plant (R)	1 µL	115.2 µL
Final Volume	100 µL	11,520 µL

   Add 100 µL of the Bead-Beating Master Mix per well of Tube Strips.
4. Seal Tube Strips tightly with Cap Strips.
5. Perform sample hogenization.

   Note: Depending on the rigidity of the specific tissue, beating time needs to be adjusted to receive a homogenous tissue paste.
6. Centrifuge Tube Strips for 1 minute at 1,000 x g with the Cap Strips attached to collect the lysate at the bottom of the well. Remove Cap Strips.
7. For individual loading, transfer 100 µL 96 Plant Lysis Buffer (LB) and 5 µL SmartLyse™ P Protease (P) to each well. Otherwise, prepare Lysis Master Mix with 20% excess volume for the number of samples (see table). at maximum speed.

Number of samples	1	96 (+20%)
Plant Lysis Buffer LB	100 µL	11,520 µL
SmartLyse™ P Protease P	5 µL	576 µL
Final Volume	105 µL	12,096 µL

Add 105 µL of the Lysis Master Mix per well of Tube Strips.

Note: If sample type is strongly absorbing liquid (e.g. freeze-dried material, seeds etc.), the amount of added Plant Lysis Buffer LB needs to be increased to 200 µL.

8. Seal Tube Strips tightly with Cap Strips.
9. Place the Tube Strips in the thermal shaker and incubate at 60 °C for 30 minutes with maximum agitation.
   If using Heating Block or heat chamber, vortex halfway through incubation time to re-suspend, and return to incubation.

Meanwhile during lysis, proceed with "Preparation of Purification Plate".

10. After incubation at 60 °C, increase the temperature to 80 °C and incubate for additional 10 minutes with maximum agitation.
11. After having performed lysis, detach the Cap Strips from the incubated Tube Strips and add 25 µL Clearing Solution P CS to wells of the Tube Strips. Mix by pipetting up and down. The sample will become cloudy.
12. Seal Tube Strips tightly with Cap Strips.
13. Centrifuge Tube Strips for 3 minutes at maximum speed.

Preparation of Purification Plate

14. Carefully detach the lower and upper sealing foils from the Purification Plate.

    Note: If the purification plate was not shipped or stored upright, resin may stick to the upper foil. In this case, horizontally shake plate until resin is removed from upper foil.

15. Plate preparation: Place the Purification Plate on top of the Conditioning Plate (a 96-deep well plate with a minimum well volume of 800 µL, not supplied) and centrifuge for 1 minute at 1,000 x g to collect the void buffer from the Purification Plate. Discard the flow-through ("void volume") collected in the Conditioning Plate (Conditioning Plate can be re-used).
16. Place conditioned Purification Plate on top of the Storage Plate for collection of purified DNA.

Purification of DNA

17. Transfer the lysis supernatant containing the DNA into the prepared Purification Plate. Important loading instructions:
    - Using the 8-channel pipette, carefully obtain the supernatant containing the DNA. Avoid any cellular debris at the bottom of the wells as it may clog the pipette tips. It is recommended to use wide-bore pipette tips for this step

      Note: Residual tissue particles may be loaded and will not interfere with purification.
    - Slowly and vertically release the supernatant onto the middle of the resin surface.
    - Do not punch pipette tip into the resin bed during loading of supernatant.
18. Centrifuge Purification Plate on top of the Storage Plate for 1 minute at 1,000 x g. The purified DNA flows through the plate into the Storage Plate. Discard the Purification Plate.

The collected DNA can be used immediately or kept at 2-8 °C or for long-term storage at –20 °C. For spectrophotometric analysis, use the 1x Tris Buffer T supplied with the kit.

Precautions and Disclaimer

This product is for Research use only. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Product Ordering

Purchase online at SigmaAldrich.com/products.

Description	Qty	Catalogue No.
GenElute™-E Single Spin Plant DNA 96 Kit	2	EC596-2EA
	8	EC596-8EA
GenElute™-E Single Spin Plant DNA Kit	10	EC500-10RXN
	50	EC500-50RXN
	250	EC500-250RXN

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