Quick Start

GenElute™-E Single Spin Tissue DNA Kit

For Purification of Genomic DNA from Tissue Samples

EC300

Quick-Start Protocol

(See Standard Protocol for detailed instructions.)

Lysis

- Add 1 – 20 mg of tissue to reaction tube.
- Add 90 µL Tissue Lysis Buffer (LB).
- Add 5 µL SmartLyse™ T Protease Mix (P).
- Incubate 30 minutes at 60 °C, maximum agitation.
- Incubate 10 minutes at 80 °C, maximum agitation.
- Add 1 µL RNase A Tissue Ⓡ. Vortex to mix.
- Incubate at room temperature for 2 minutes.
- Add 10 µL Clearing Solution T (CS) and vortex shortly.
- Centrifuge 2 minutes at maximum speed.

Spin Column preparation

(during 60 °C and 80 °C incubation)

- Vortex GenElute™-E Spin Column and place in a 2 mL tube.
- Let stand for 10–20 minutes.
- Loosen screw cap of Spin Column.

  Optional: Punch a hole in the cap with the GenElute™-E Cap Puncher.
- Snap off bottom closure.
- Place Spin Column back into 2 mL tube.
- Centrifuge 1 minute at 1,000 x g to collect Spin Column buffer.
- Place Spin Column in a 1.5 mL tube.

Purification of DNA

- Transfer lysate supernatant (maximum 100 µL) to prepared Spin Column.
- Centrifuge 1 minute at 1,000 x g to collect DNA.
- Collected DNA is ready to use.

Intended Use

For single-step purification of genomic DNA from tissue samples. This protocol has been developed for 1 – 20 mg human and animal tissue samples. 10 mg is generically recommended (for certain species, optimization of input amount may be required). For high DNA content (e g., spleen, liver, kidney): 5 mg is recommended. For low DNA content (e g., muscle, cartilage): 20 mg is recommended.

Storage and Stability

Store SmartLyse™ T Protease (P) and RNase A Tissue Ⓡ at 2-8 ºC. The remaining components should be stored at room temperature. Use the kit within 12 months of receipt.

Materials and Equipment Needed

Kit Contents

- Tissue Lysis Buffer (LB)
- SmartLyse™ T Protease (P)
- Clearing Solution T (CS)
- RNase A Tissue Ⓡ
- 1x Tris Buffer Ⓣ
- Spin Columns

Not Supplied with Kit

- Microcentrifuge with rotor for 1.5 mL and 2 mL reaction tubes.

  Important: Set centrifuge to relative centrifugal force, rcf (x g). If needed, calculate equivalent rpm by the formula:

  rpm = 1,000 x √(g/(1.12 x r)),
  where r = radius of rotor in mm
  and g is the required g-force.
- 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 and 200 µL scales, corresponding pipet tips.
- One reaction tube (1.5 mL) per sample for the lysis step.
- One reuseable reaction tube (2 mL) per sample for Spin Column preparation.
- One reaction tube (1.5 mL) per sample for collection of the purified DNA.

Preparation Before Starting

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

Standard Protocol

Lysis

1. Add 1 – 20 mg of tissue sample to reaction tube.

   Note: To avoid degradation, keep samples on ice or in a cooling block during sample loading.

   - If possible, cut tissue into small pieces to speed up lysis.
   - For stabilized tissue samples briefly rinse with water to remove traces of stabilization solution before adding samples to the reaction tube.

2. For each sample, add 90 µL Tissue Lysis Buffer LB and 5 µL SmartLyse™ T Protease P. If working with more than two samples, prepare a Lysis Master Mix with 10% excess volume for the number of samples (see table).

   Lysis Master Mix

Number of samples	1	6 (+10%)	12 (+10%)
Tissue Lysis Buffer LB	90 µL	594 µL	1,188 µL
SmartLyse™ T Protease P	5 µL	33 µL	66 µL
Final Volume	95 µL	627 µL	1,254 µL

3. Place the reaction tube(s) 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 "Spin Column Preparation".

Note: If samples are not completely lysed after the time period described above, continue with the next step. Residual cellular debris will not interfere with the purification performance.

Note: For some tissue types, lysis is already complete after 15 minutes. Therefore, this step may be shortened accordingly.

4. After incubation at 60 °C, increase the temperature to 80 °C and incubate for additional 10 minutes with maximum agitation.

   Optional: After having performed lysis, add 1 µL RNase A Tissue ® to each lysed sample and vortex for 3 seconds. Incubate for 2 minutes at room temperature to remove RNA traces.

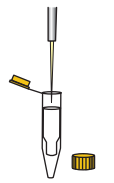
5. Add 10 µL Clearing Solution T CS. Vortex for 3 seconds. The sample will become cloudy.
6. Centrifuge for 2 minutes at maximum speed.

Spin Column Preparation

7. Vortex the GenElute™-E Spin Column briefly and place into a 2 mL reaction tube. Let stand for 10 to 20 minutes.
8. Loosen the screw cap of the Spin Column and snap off bottom closure of the Spin Column. The screw cap must stay loosened half a turn to avoid generation of a vacuum. Place the Spin Column back into the 2 mL reaction tube.
9. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing Spin Column buffer.
10. Place the prepared GenElute™-E Spin Column into a new 1.5 mL reaction tube for collection of the purified DNA and place back into the rack.

Purification of DNA

11. Transfer a maximum of 100 µL of lysis supernatant containing the DNA into the prepared GenElute™-E Spin Column as illustrated:



   - Open cap and pipet the sample slowly (5 sec) onto the center of the resin bed of the prepared Spin Column.
   - Close screw cap and loosen again half a turn.

     Important: Do not re-close the screw cap of the Spin Column completely.

   Note: During loading of lysate, do not touch the resin bed with your pipette tip. Residual cellular debris may be loaded and will not interfere with purification.

12. Centrifuge for 1 minute at 1,000 x g. The purified DNA flows through the Spin Column into the 1.5 mL storage tube. Discard the Spin Column.

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 Ⓣ supplied with the kit.

Cap Puncher Protocol

Lysis

1. Perform Standard Protocol steps 1-7.

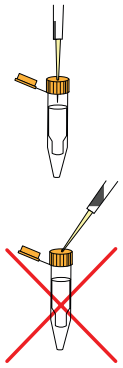
Spin Column Preparation

8. Vortex the GenElute™-E Spin Column briefly and place into a 2 mL reaction tube. Let stand for 10 to 20 minutes.
9. Use of the Cap Puncher: Punch a hole into the Spin Column cap and lift the Spin Column together with the Cap Puncher out of the 2 mL collection tube. Snap off bottom closure of the Spin Column and detach the Cap Puncher by twisting clockwise while pulling out. Place the punched Spin Column back into the 2 mL reaction tube.
10. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the Spin Column buffer.
11. Place the prepared GenElute™-E Spin Column into a new 1.5 mL reaction tube for collection of the purified DNA and place back into the rack.

Purification of DNA

12. Transfer a maximum of 100 µL of lysis supernatant containing the DNA into the prepared GenElute™-E Spin Column:
    - Insert pipet tip vertically through the hole in the Spin Column cap.
    - Pipet the sample slowly (5 sec) into the Spin Column.

    Note: Residual cellular debris may be loaded and will not interfere with purification.



13. Centrifuge for 1 minute at 1,000 x g. The purified DNA flows through the Spin Column into the 1.5 mL storage tube. Discard the Spin Column.

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 Ⓣ supplied with the kit.

Product Ordering

Purchase online at SigmaAldrich.com/products.

Description	Qty	Catalogue No.
GenElute™-E Single Spin Tissue DNA Kit	10	EC300-10RXN
	50	EC300-50RXN
	250	EC300-250RXN
GenElute™-E Single Spin Tissue DNA 96 Kit	2 EA	EC396-2EA
	8 EA	EC396-8EA
GenElute™-E Single Spin Cap Puncher	1 EA	EC9999-1EA

Precautions and Disclaimer

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

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