Quick Start

GenElute™-E Single Spin Tissue DNA 96 Kit

For 96-Well Purification of Genomic DNA from Tissue Samples

EC396

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

Lysis

Add 1–20 mg of tissue sample per well of Lysis Plate.

- Prepare Lysis Master Mix, add 135 µL per well of Lysis Plate.

Number of samples	1	96 (+20%)
Tissue Lysis Buffer (LB)	130 µL	14,976 µL
SmartLyse™ T Protease (P)	5 µL	576 µL
Final Volume	135 µL	15,552 µL

- Seal Lysis Plate tightly with Adhesive Foil. Incubate 30 minutes at 60 °C, maximum agitation.
- Incubate 10 minutes at 80 °C, maximum agitation.
- Remove Adhesive Foil. Prepare RNase Digest Master Mix, add 16 µL per well of Lysis Plate.

Number of samples	1	96 (+20%)
Clearing Solution T (CS)	15 µL	1,728 µL
RNase A Tissue ®	1 µL	115.2 µL
Final Volume	16 µL	1,843.2 µL

- Mix by pipetting. Centrifuge Lysis Plate 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 Lysis Plate 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 tissue samples. This protocol has been developed for 1 mg-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 recommended. For low DNA content (e g., muscle, cartilage): 20 mg recommended.

Storage and Stability

Store SmartLyse™ T Protease (P), RNase A Tissue ® and Purification Plates 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

- Lysis Plate: 96-well plate for lysis of tissue samples in a 96-well thermal shaker.
- 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 during lysis.
- Reagents:
  - Tissue Lysis Buffer (LB)
  - SmartLyse™ T Protease (P)
  - RNase A Tissue ®
  - Clearing Solution T (CS)
  - 1x Tris Buffer Ⓣ.

Not Supplied with Kit

- 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 x √(g/(1.12 x 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 are being processed.

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 1–20 mg of tissue sample per well of Lysis Plate.

   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 individual loading, transfer 130 µL Tissue Lysis Buffer (LB) and 5 µL SmartLyse™ T Protease (P) to each well. Otherwise, prepare Lysis Master Mix with 20% excess volume for the number of samples (see table).

Number of samples	1	96 (+20%)
Tissue Lysis Buffer (LB)	130 µL	14,976 µL
SmartLyse™ T Protease (P)	5 µL	576 µL
Final Volume	135 µL	15,552 µL

   Add 135 µL of the Lysis Master Mix per well of Lysis Plate.
3. Seal Lysis Plate tightly with Adhesive Foil.
4. Place the Lysis Plate 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.

   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.

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

5. After incubation at 60 °C, increase the temperature to 80 °C and incubate for additional 10 minutes with maximum agitation.
6. After having performed lysis, detach the Adhesive Foil from the incubated Lysis Plate and add 15 µL Clearing Solution T CS to well of the Lysis Plate. Mix by pipetting up and down. The sample will become cloudy.
   Note: Although RNase digestion is not necessary for most tissue samples, certain tissues contain higher RNA amounts which can be eliminated with an RNase digest. In these cases, prepare a RNase Digest Master Mix with RNase A Tissue ® and Clearing Solution T CS:

Number of samples	1	96 (+20%)
Clearing Solution T CS	15 µL	1,728 µL
RNase A Tissue ®	1 µL	115.2 µL
Final Volume	16 µL	1,843.2 µL

7. Add 16 µL of the RNase Digest Master Mix per well of Lysis Plate. Mix by pipetting up and down and incubate at room temperature for 2 minutes.
8. Centrifuge Lysis Plate for 3 minutes at maximum speed.

Preparation of Purification Plate

9. 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.
10. 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).
11. Place conditioned Purification Plate on top of the Storage Plate for collection of purified DNA.

Purification of DNA

12. Transfer a maximum of 100 µL per well of lysis supernatant containing the DNA into the prepared Purification Plate (maximum of 100 µL per well). 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 sample precipitate 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.
13. Centrifuge Purification Plate on top of the Storage Plate for 1 minute at 1,000 x g. The purified DNA flows through the well 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 Ⓣ supplied with the kit.

Product Ordering

Purchase online at SigmaAldrich.com/products.

Description	Qty	Catalogue No.
GenElute™-E Single Spin Tissue DNA 96 Kit	2 EA	EC396-2EA
	8 EA	EC396-8EA
GenElute™-E Tissue Stabilizer	100 mL	EC111-100ML
	500 mL	EC111-500ML
GenElute™-E Single Spin Tissue DNA Kit	10	EC300-10RXN
	50	EC300-50RXN
	250	EC300-250RXN

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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