Quick Start

GenElute™-E Single Spin Blood DNA 96 Kit

For 96-Well Purification of Genomic DNA from Liquid Blood Samples

EC196

Quick-Start Protocol

(See Standard Protocol for detailed instructions.)

Lysis

- Prepare Lysis Master Mix, add 60 µL of the Lysis Master Mix per well of Lysis Plate.

Number of samples	1	96 (+20%)
Blood Lysis Buffer (LB)	50 µL	5,760 µL
SmartLyse™ B Protease (P)	10 µL	1,152 µL
Final Volume	60 µL	6,912 µL

- Add up to 60 µL of blood sample per well of Lysis Plate. Seal with Adhesive Foil.
- Incubate 30 minutes at 60 °C, maximum agitation.
- Incubate 10 minutes at 80 °C, maximum agitation.
- Detach Adhesive Seal and add 10 µL Clearing Solution B (CS). 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 the 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 liquid blood samples. This protocol has been developed for up to 60 µL of human or animal whole blood (EDTA-, Citrate- or Heparin-stabilized) or buffy coats. Erythrocytes from non-mammals (e.g. birds, fish, reptiles) contain DNA. Here, a volume of 5-10 µL of blood per purification is recommended.

Storage and Stability

Store SmartLyse™ B Protease (P) and Purification Plates at 2-8 ºC. The remaining components should be stored at room temperature. Use the kit within 12 months of receipt.

Kit Contents

- Lysis Plate: 96-well plate for lysis of blood 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 Foil for plate sealing.
- Reagents:
  - Blood Lysis Buffer (LB)
  - Clearing Solution B (CS)
  - SmartLyse™ B Protease (P)
  - 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 centrifuge.

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 pipet for 200 µL scale, corresponding pipet tips.
- Troughs for Master Mix preparation 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. For each sample, transfer 50 µL Blood Lysis Buffer LB and 10 µL SmartLyse™ B Protease Mix P.

   If working with more than two samples, prepare a Lysis Master Mix with 20% excess volume for the number of blood samples (see table).

Number of samples	1	96 (+20%)
Blood Lysis Buffer LB	50 µL	5,760 µL
SmartLyse™ B Protease P	10 µL	1,152 µL
Final Volume	60 µL	6,912 µL

   Add 60 µL of the Lysis Master Mix per well of Lysis Plate.

2. Add up to 60 µL of blood sample per well of the Lysis Plate.
2. Seal Lysis Plate tightly with Adhesive Foil.
3. 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.

   Lysis time can be shortened to 15 minutes without loss in PCR performance but A260/A230 purity ratio may be lowered.

   Note: Agitation during lysis is mandatory for blood samples (in contrast to other sample types).

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

4. After incubation at 60 °C, increase the temperature to 80 °C and incubate for additional 10 minutes with maximum agitation.
5. After having performed lysis, detach the Adhesive Foil from the incubated Lysis Plate and add 10 µL Clearing Solution B CS to each well of the Lysis Plate. Mix by pipetting up and down. The sample will become cloudy.

   Note: Usually, the addition of RNase is not required due to endogenous RNases. If RNA needs to be stringently degraded, add 1 µL RNase before the addition of Clearing Solution B CS and incubate for 2 minutes at room temperature.

   Note: For extraction from buffy coat, pipet the lysate up and down 10 times before proceeding to centrifugation.
6. Centrifuge Lysis Plate for 3 minutes at maximum speed.

Preparation of Purification Plate

7. 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.
8. 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).
9. Place conditioned Purification Plate on top of the Storage Plate for collection of purified DNA.

Purification of DNA

10. 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 blood 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.

11. Centrifuge the Purification Plate on top of the Storage Plate for 1 minute at 1,000 x g. The purified DNA flows through the Purification 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 Ⓣ 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 Blood DNA 96 Kit	2	EC196-2EA
	8	EC196-8EA
GenElute™-E Single Spin Blood DNA Kit	10	EC100-10RXN
	50	EC100-50RXN
	250	EC100-250RXN
GenElute™-E Single Spin Blood DNA High Yield Kit	10	EC200-10RXN
	50	EC200-50RXN
	250	EC200-250RXN

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