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Quick Start

GenElute™-E Single Spin Blood DNA Kit for Dried Blood Spots

For Purification of Genomic DNA from Dried Blood Samples

EC100

Quick-Start Protocol

(See Standard Protocol for detailed instructions.)

Lysis

- Add 100 µL Blood Lysis Buffer LB.
- Add 10 µL SmartLyse™ B Protease Mix P.
- Add 1 to 5 punched-out dried blood spots, vortex briefly.
- Incubate 30 minutes at 60 °C, maximum agitation.
- Incubate 10 minutes at 80 °C, maximum agitation.
- Add 10 µL Clearing Solution B CS and vortex shortly.
- Centrifuge 2 minutes at maximum speed.

Column Preparation (during 60 °C and 80 °C incubation)

- Vortex 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 Cap Puncher.
- Snap off bottom closure.
- Place Spin Column back into 2 mL tube.
- Centrifuge 1 minute at 1,000 x g to collect column buffer.
- Place column in a 1.5 mL tube.

Purification of DNA

- Transfer lysate supernatant (maximum 100 µL).
- 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 dried blood samples. This protocol has been developed for 1 to 5 punched-out dried blood spots (3 mm).

Precautions and Disclaimer

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

Storage and Stability

Store SmartLyse™ B Protease P at 2-8 °C. The remaining components should be stored at room temperature. Use the kit within 12 months of receipt.

Materials and Equipment Needed

Kit Contents

- Blood Lysis Buffer LB
- SmartLyse™ B Protease P
- Clearing Solution CS
- 1x Tris Buffer T
- Spin Columns

Not Supplied in 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:

  $rpm = 1{,}000 \times \sqrt{g/(1.12 \times r)}$,
  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.

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- 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 reusable reaction tube (2 mL) per sample for 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. For each sample, transfer 100 µL Blood Lysis Buffer LB and 10 µL SmartLyse™ B Protease Mix P to a 1.5 mL reaction tube. If working with more than two samples, prepare a Lysis Master Mix with 10% excess volume for the number of blood samples (see table).

Lysis Master Mix:

Number of samples	1	6 (+10%)	12 (+10%)
Blood Lysis Buffer LB	100 µL	660 µL	1320 µL
SmartLyse™ B Protease P	10 µL	66 µL	132 µL
Final Volume	110 µL	762 µL	1452 µL

Add 110 µL of the Lysis Master Mix to each 1.5 ml reaction tube.

2. Add 1 to 5 punched-out dried blood samples. Vortex briefly.
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.

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

Meanwhile during lysis, proceed with "Column Preparation".

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, add 10 µL Clearing Solution B CS to each sample and vortex 3 sec. 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.

6. Centrifuge for 2 minutes at maximum speed.

Column Preparation

7. Vortex the 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. Remove column from reaction tube and snap off the bottom of the 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 the column buffer.
10. Place the prepared Spin Column into a new 1.5 mL reaction tube for collection of the purified DNA and place back into the rack.

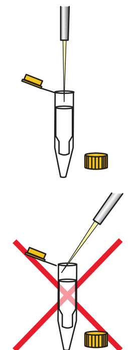
Purification of DNA

11. Transfer a maximum of 100 µL of lysis supernatant containing the DNA into the prepared 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 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 (optional)

The Spin Column caps contain resealing valves that prevent any sample contamination through the cap after it has been punched as well as any contamination from outside the column and from DNA preparations performed in parallel. During sample loading, make sure to pierce the valve with the pipet tip; this is indicated by a slight pressure release.

Lysis

1. Perform steps 1-6 from Standard Protocol.

Column Preparation

7. Vortex the Spin Column briefly and place into a 2 mL reaction tube.

   Let stand for 10 to 20 minutes.

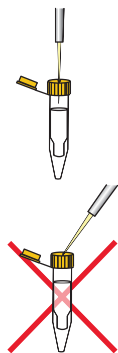
8. Use of the Cap Puncher: Punch a hole into the column cap and lift the column together with the Cap Puncher out of the 2 mL collection tube. Snap off bottom closure of the column and detach the Cap Puncher by twisting clockwise while pulling out. Place the punched Spin Column back into the 2 mL reaction tube.
9. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the column buffer.
10. Place the prepared 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 lysis supernatant containing the DNA into the prepared Spin Column as illustrated:

    Insert pipet tip vertically through the hole in the column cap until forced through the lid valve (slight pressure release; otherwise, re-apply the pipet tip). Pipet the sample slowly (5 seconds) into the column.

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



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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 Blood DNA Kit	10	EC100-10RXN
	50	EC100-50RXN
	250	EC100-250RXN
GenElute™-E Single Spin Cap Puncher	1 EA	EC9999-1EA

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