Quick Start

GenElute™-E Single Spin Cell Culture DNA Kit

For single-step purification of genomic DNA from cultured cells

EC400

Quick-Start Protocol

(See Standard Protocol for detailed instructions)

Lysis

- Transfer up to 1 x 10^6 cultured cells.
- Centrifuge 1 minute at 2,000 x g to pellet cells. Discard supernatant.
- Add 55 µL Cell Lysis Buffer LB.
- Add 25 µL SmartLyse™ C Protease Mix P.
- Pulse-vortex to resuspend the pelleted cells.
- Incubate 10 minutes at 60 °C, maximum agitation.
- Add 25 µL Clearing Solution C CS and 1 µL RNase A Cell Ⓡ.
- Vortex vigorously with four pulses of 10 seconds each.
- Incubate for 2 minutes at room temperature.
- Centrifuge 2 minutes at maximum speed.

Column preparation (during 60 °C incubation)

- Vortex Spin Column and place in a 2 mL tube.
- Let stand for 5 to 10 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 cultured cells. This protocol has been developed for up to 1 x 10^6 human or animal cells.

Storage and Stability

Store SmartLyse™ C Protease P and RNase A Cell Ⓡ 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

- Cell Lysis Buffer LB
- SmartLyse™ C Protease P
- Clearing Solution C CS
- RNase A Cell Ⓡ
- 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 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 2,000 x g (cell pelleting) or 1,000 x g (column preparation).

Standard Protocol

Lysis

1. Harvest cells (up to 1x10^6 cells) by centrifugation at 2,000 x g for 1 minute in a 1.5 mL reaction tube and remove supernatant carefully. Discard supernatant.

   Important: Make sure that the cell pellet is retained during this step.

2. For each sample, add 55 µL Cell Lysis Buffer LB and 25 µL SmartLyse™ C Protease Mix 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%)
Cell Lysis Buffer LB	55 µL	363 µL	726 µL
SmartLyse™ C Protease P	25 µL	165 µL	330 µL
Final Volume	80 µL	528 µL	1,056 µL

   Add 80 µL of the Lysis Master Mix to a 2 mL reaction tube.

3. Pulse-vortex to resuspend cell pellet.
4. Place the reaction tube(s) in the thermal shaker and incubate at 60 °C for 10 minutes with maximum agitation.

   If using Heating Block or heat chamber, vortex halfway through incubation time to re-suspend, and return to incubation.

5. After having performed lysis, add 10 µL Clearing Solution C CS and 1 µL RNase ® to the sample. Vortex vigorously with four pulses of 10 seconds each. The sample becomes cloudy.
6. Incubate for 2 minutes at room temperature.
7. Centrifuge for 2 minutes at maximum speed.

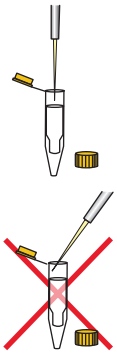
Column Preparation

8. Vortex the Spin Column briefly and place into a 2 mL reaction tube. Let stand for 5 to 10 minutes.
9. Loosen the screw cap of the Spin Column and snap off bottom closure 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.
10. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the column buffer.
11. 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

12. 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 sample particles 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 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 T supplied with the kit.

Cap Puncher Protocol

Lysis

1. Perform Standard Protocol steps 1-7.

Column Preparation

8. Vortex the GenElute™-E Spin Column briefly and place into a 2 mL reaction tube. Let stand for 5 to 10 minutes.
9. 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.
10. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the 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 Spin Column as illustrated:

- Insert pipet tip vertically through the hole in the column cap.

- Pipet the sample slowly (5 sec) into the 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 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 Cell Culture DNA Kit	10	EC400-10RXN
	50	EC400-50RSN
	250	EC400-250RXN
GenElute™-E Single Spin Cap Puncher	1 EA	EC9999-1EA

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