User Manual

GenElute™-E Single Spin Blood DNA Kit

For Purification of Genomic DNA from Liquid Blood Samples and Dried Blood Spots

EC100

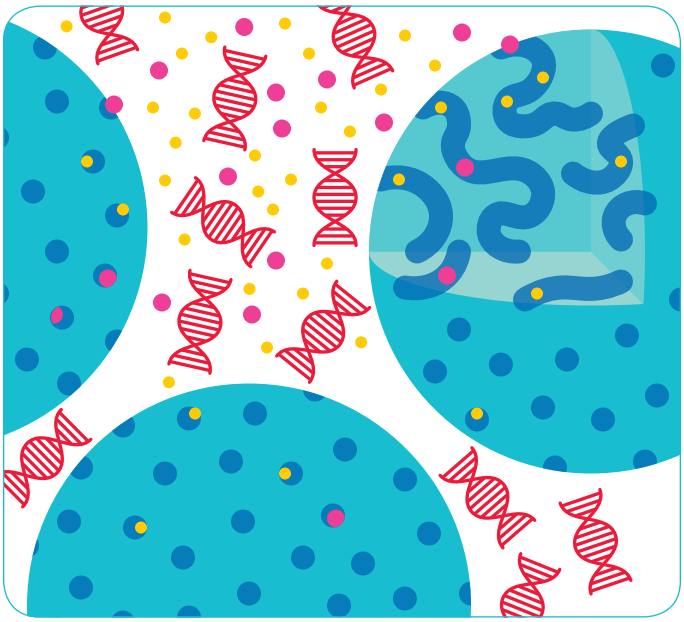
Sigma-Aldrich®

What's Inside:

For research use only.

Introduction

GenElute™-E Single Spin Kits are a nucleic acid purification system that eliminates the need for high salt binding and ethanol wash steps, yielding DNA and RNA preparations with fewer impurities for more robust results. GenElute™-E DNA and RNA purification kits employ a **negative chromatography** method dependent on size exclusion to separate large DNA and RNA nucleic acid molecules from smaller protein, lipid, and ionic components in cell, tissue, blood, and other samples.



Using negative chromatography, Single Spin columns efficiently absorb and retain sample contaminants while allowing nucleic acids to flow through the column, reducing the number of steps and plastic materials required for purification. The key is the novel lysis that allows negative chromatography to be used for high quality nucleic acid purification.

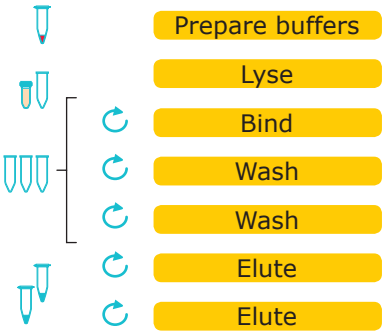
Three key advantages over silica:

- Simplified workflow
- Superior performance
- Waste reduction

A Simplified Workflow

Purification in one spin, eliminating all wash steps and reducing tube handling for more efficient, safer sample processing.

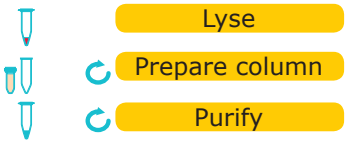
Silica Procedure



Multiple spins
and tube transfers

Lysis
45 minutes to overnight
Purification
45 minutes hands-on time
6 centrifugation steps

GenElute™-E Single Spin Technology



- ✓ Reduced time
- ✓ Reduced handling
- ✓ Reduced waste

Lysis
10 to 40 minutes
Purification
3 minutes hands-on time
1 centrifugation step

Reduced Waste for a Better Environment

With fewer plastic tubes and no hazardous liquids, GenElute™-E DNA and RNA purification kits provide an eco-friendly alternative to silica-based purification.

GenElute™-E purification kits greatly reduce the amounts of plastic-based components packaged with each kit and consumed while executing protocols in the lab. All tedious binding and washing steps associated with silica-based procedures are omitted, with no use of hazardous materials such as chaotropic salts or organic solvents that require special disposal. Plastic waste is reduced by 55% compared to a common silica kits, resulting in disposal cost savings and reduced environmental impact.

GenElute™-E Single Spin nucleic acid purification kits provide easier workflows for DNA and RNA isolation, better nucleic acid quality with fewer impurities, and reduced plastic and hazardous waste disposal compared to silica bind-wash-elute spin prep kits.

GenElute™-E Single Spin Purification supports:

- Significantly reduced plastic waste
- No hazardous bind and wash steps
- Responsible and sustainable nucleic acid purification
- Disposal cost savings

GenElute™-E kits

Supplier X

Specifications

Sample Input	Up to 60 µl or 2-5 dried blood spots
Sample Type	Human and animal whole blood
Sample Condition	Fresh, frozen, stabilized
Required time after lysis	2 minutes
Purified Nucleic Acid	DNA > 200 bp
Elution Volume	90-110 µl
The purified genomic DNA is ready for immediate use in downstream applications:	• Restriction digestions • PCR and qPCR • Southern blots • Sequencing reactions

Intended Use

For single-step purification of genomic DNA from liquid blood samples or dried blood spots. This protocol has been developed for up to 60 µL (fresh, stabilized or frozen) of human or animal whole blood (EDTA-, Citrate- or Heparin-stabilized) or buffy coats. This protocol has also been developed for 1 to 5 punched-out dried blood spots (3 mm).

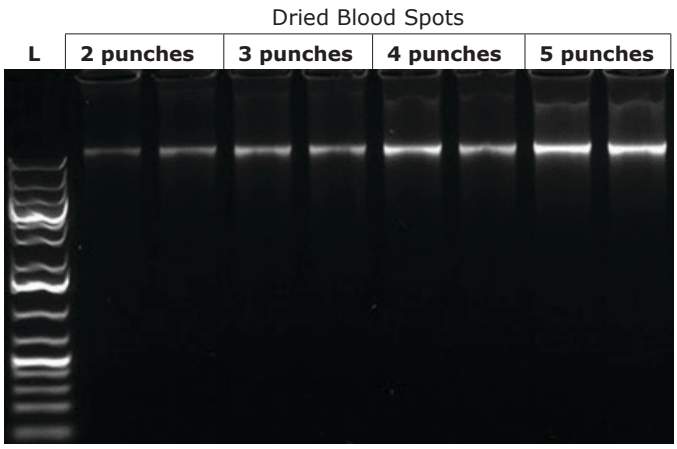
Typical Results

Table 1

Sample Type	Typical Yield (µg/200µl)
EDTA-Stabilized Human	2-10
Citrate-Stabilized Human	2-4
Heparin-Stabilized Human	1-3
Buffy Coat	5-25
Mouse Blood	3-5
Chicken Blood	3-15
Tilapia Blood	5-10
Dried Blood Spots	1-15

Typical yields reflect µg of gDNA per 200 µl of initial sample. Due to sample variability, results may vary.

Figure 1



Gel electrophoresis results of duplicate isolations from 2-5 punches of dried blood spots using GenElute™-E Single Spin Blood DNA Kit.

Table 2

	Average Concentration (ng/µl)	Average Quality (260/280)	Average Mass (µg)
Buffy coat	95.77	1.67	9.58
EDTA-stabilized blood	11.08	1.64	1.11
Citrate-stabilized blood	8.02	1.54	0.80
Heparin-stabilized blood	4.90	1.33	0.49

Average spectrophotometric results of from eight replicates of gDNA isolation from buffy coats, EDTA-stabilized blood, and Citrate-stabilized blood and from three replicates of gDNA isolation from Heparin-stabilized blood using GenElute™-E Single Spin Blood DNA Kit.

Storage and Stability

Kit Storage

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

Sample Storage and Variability

Nucleic acid degrades over time, potentially leading to reduced fragment length and overall yield. Therefore, it is best if samples are acquired from fresh material. Often when working with samples, this is not feasible. Stabilizing the sample through the addition of stabilizing reagents can assist in improving results. Be aware that the DNA yield to be expected is donor-dependent.

Disposal

GenElute™-E kits adhere to the principles of "SMASH Packaging", our plan that drives improvement in the sustainability of our packaging through less packaging, more sustainable materials and easier recycling.

The box and insert material comes from sustainably managed forests and/or more than 70% of recycled content. The kit componet bags are composed of starch-based, compostable material. Please recycle.

Kit components exposed to samples should be disposed of with biological waste. Other kit materials should be disposed of according to all applicable international, federal, state, and local regulations.

Materials and Equipment Needed

Kit Contents

- Blood Lysis Buffer (LB)
- SmartLyse™ B Protease (P)
- Clearing Solution B (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.
- 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.
- GenElute™-E Cap Puncher (optional)

Preparation before starting

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

Liquid Blood Sample Standard Protocol

Lysis

1. For each sample, transfer 50 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)	50 µL	330 µL	660 µL
SmartLyse™ B Protease (P)	10 µL	66 µL	132 µL
Final Volume	60 µL	396 µL	792 µL

   Add 60 µL of the Lysis Master Mix to each 1.5 mL reaction tube.

2. Add up to 60 µL of blood sample. 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 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 seconds. 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 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 for 2 minutes at maximum speed.

Column Preparation

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

8. Let stand for 10 to 20 minutes.

9. 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.

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.

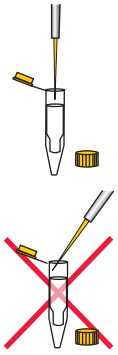
Purification of DNA

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 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 (T) supplied with the kit.

Dried Blood Spot 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.

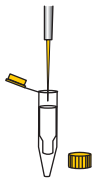
8. Loosen the screw cap of the spin column. Remove column from reaction tube and snap off the bottom of the column. The screw cap must stay loosened half a turn to avoid generation of a vacuum. Place the Spin Column back into the 2 mL reaction tube.

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 (T) supplied with the kit.

Cap Puncher Protocol

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 Protocols and continue directly to Column Preparation.

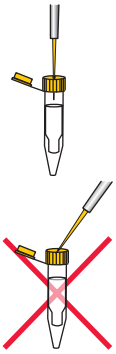
Column Preparation

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 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 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.

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.

Troubleshooting

Problem	Probable Cause	Solution
Low yield	Individual samples have inherent variability Be aware that the DNA yield to be expected is donor-dependent. Optimization needs to be performed by the user to validate for their sample type.	Degraded DNA fragments below < 60 bp are depleted during purification. Using fresh samples stored under appropriate conditions or stabilizing the samples will help to mitigate low sample yields.
	Using too much sample may result in overloading the column's capacity for separation.	Use the recommended sample load. Optimization needs to be performed by the user to validate for their sample type.
	Using too little of sample may result in low sample yields.	Use the recommended sample load. Optimization may need to be performed by the user if their sample type is low yielding.
	Small, possibly degraded, DNA-fragments (< 60 bp) and/or RNA (if RNase was used) are removed during purification. Since these components are co-purified with silica-based kits, there may be artificially lower oD 260 readings with GenElute™-E kits. Thus, the calculation of sample concentration and subsequent yield may appear lower.	Quantitation by measuring signal intensity of bands via gel electrophoresis fragment separation, using a fragment analyzer, or comparing qPCR Ct values will provide a more reliable measurement of full-length gDNA.
	Centrifugation speeds and spin times have been optimized to acquire the fraction of sample containing the nucleic acid.	Verify that centrifugation was performed under the recommended conditions.
	Incomplete lysis of the sample may lead to reduced yields. Lysis times may need to be extended depending on the sample type.	Verify that centrifugation was performed under the recommended conditions.
	If the column preparation steps were performed incorrectly, then the separation resin will be packed incorrectly.	Verify that the preparation steps for the column were performed according to the protocol.
	When performing the Standard Protocol, without the use of the GenElute™-E Single Spin Cap Puncher, the cap may have been left untightened.	Verify that the spin column cap of the column is loosened half a turn to avoid vacuum generation.
Low sample volume	Loading too low of sample or too high of sample may result in sample volume loss. The loaded sample volume is required to be within the recommended range as that volume is required to displace the column buffer.	If the sample volume available to be loaded onto the column is below the recommended range, as may occur with dehydrated sample types, then bring the sample within the recommended range using lysis buffer. If the sample volume available to be loaded onto the column is above the recommended range, then only load up to the recommended volume.
	Blocking of pipette tips by lysate debris during aspiration of the supernatant may result in a lower transfer volume and, consequently, in a lower DNA yield.	Loading of debris onto the column will not impact the ability of the column to purify the nucleic acid from the sample. However, avoid aspirating the debris into the pipette tip or use wide-bore tips to aspirate if the sample has an abundance of debris to prevent clogging.

260/230 ratios appear to be "too low."	In some cases, the 260/230 ratios may be below the recommended range.	Downstream assays have not been shown to be compromised by lower 260/230 ratios using nucleic acid isolated using GenElute™-E kits.
RNA residues are observed.	If the optional RNase protocol is not performed, then there may be RNA observed.	Perform the optional RNase protocol. Some samples may require an extended incubation due to variability across sample types.
The purified sample has a reddish color.	Sometimes with blood samples the purified sample is reddish. In these cases, the column may be overloaded due to variations in sample type.	Reduce the amount of sample used with the kit.
Lysate leaks from the hole created by the Cap Puncher during loading	The sample needs to be loaded vertically, allowing the sample to be dispensed correctly into the column. Also, if there is not enough pressure applied using the Cap Puncher then the hole may not be large enough to load the sample.	Apply enough pressure using the Cap Puncher to create a hole and load sample vertically.
Columns with dried resin	In rare cases, the spin columns dry out during storage. This may be due to not storing the columns according to the recommended conditions.	Store GenElute™-E kits according to the recommended conditions.

Product Ordering

Description	Qty	Catalogue No.
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
GenElute™-E Single Spin Tissue DNA Kit	10	EC300-10RXN
	50	EC300-50RXN
	250	EC300-250RXN
GenElute™-E Single Spin Cell Culture DNA Kit	10	EC400-10RXN
	50	EC400-50RXN
	250	EC400-250RXN
GenElute™-E Single Spin Plant DNA Kit	10	EC500-10RXN
	50	EC500-50RXN
	250	EC500-250RXN
GenElute™-E Single Spin DNA Cleanup Kit	10	EC600-10RXN
	50	EC600-50RXN
	250	EC600-250RXN
GenElute™-E Organic Solvent DNA Cleanup	10	EC700-10RXN
	50	EC700-50RXN
	250	EC700-250RXN
GenElute™-E Single Spin RNA Cleanup Kit	10	EC800-10RXN
	50	EC800-50RXN
	250	EC800-250RXN
GenElute™-E Tissue Stabilizer	100	EC111-100ML
	500	EC111-500ML
GenElute™-E RNA Gel Loading Buffer	1	EC222-1EA
	5	EC222-5EA
GenElute™-E Single Spin Tissue DNA 96 Kit	2	EC396-2EA
	8	EC396-8EA
GenElute™-E Single Spin Plant DNA 96 Kit	2	EC596-2EA
	8	EC596-8EA
GenElute™-E Single Spin Blood DNA 96 Kit	2	EC196-2EA
	8	EC196-8EA
GenElute™-E Single Spin Cap Puncher	1	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.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

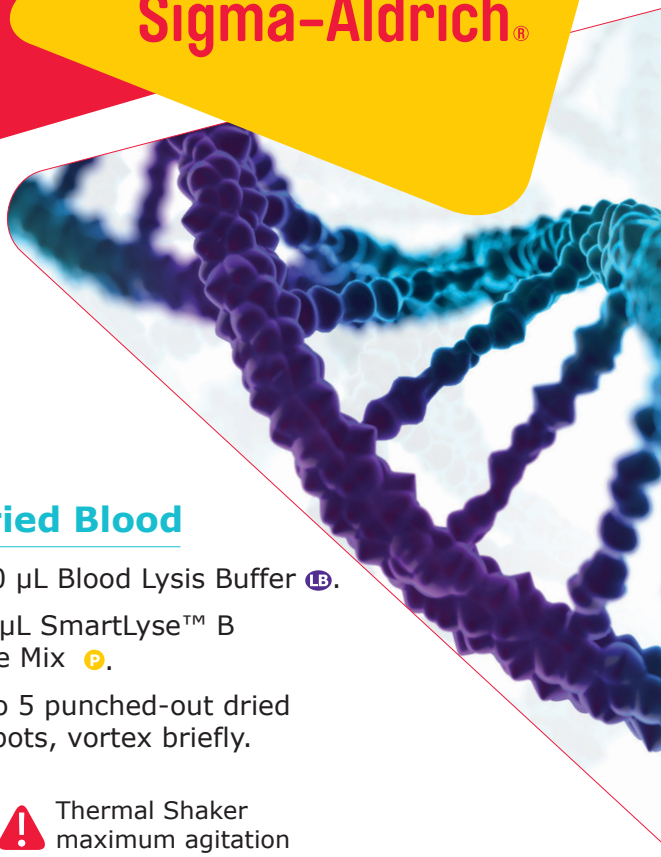
Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Assistance

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

GenElute™-E Single Spin Checklist for Blood DNA Kit



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Prepare before starting

- ☐ Prepare the reaction tubes
- ☐ Pre-heat thermal shaker to 60 °C
- ☐ Set microcentrifuge to 1,000 x g

Lysis Liquid Blood or Lysis Dried Blood

Lysis Liquid Blood	Lysis Dried Blood
☐ Add 50 µL Blood Lysis Buffer LB.	☐ Add 100 µL Blood Lysis Buffer LB.
☐ Add 10 µL SmartLyse™ B Protease Mix P.	☐ Add 10 µL SmartLyse™ B Protease Mix P.
☐ Add 60 µL blood, vortex briefly.	☐ Add 1 to 5 punched-out dried blood spots, vortex briefly.

Column prep during lysis

Incubate 30 minutes 60 °C | 10 minutes 80 °C | Thermal Shaker 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.

OR

Punch a hole in the cap with the GenElute™-E Single Spin 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.

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