User Manual

GenElute™-E Single Spin Blood DNA 96 Kit

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

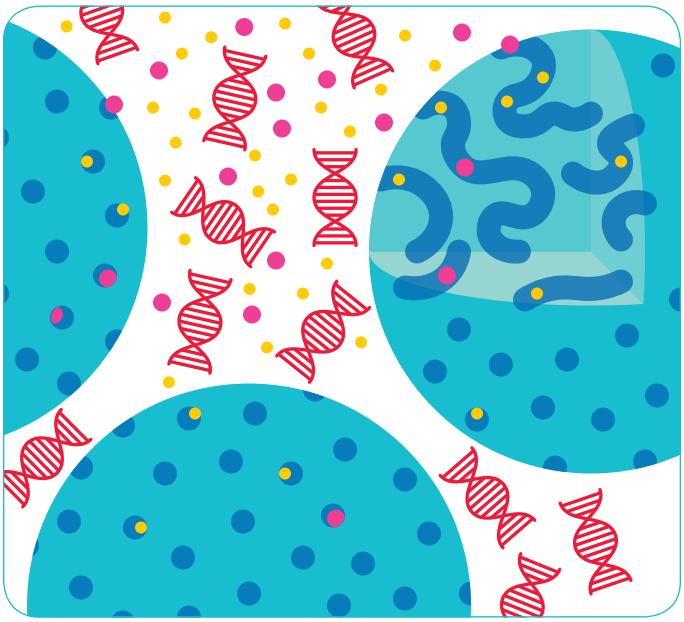
EC196

What's Inside:

For research use only.

Introduction

GenElute™-E Single Spin Kit is 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.

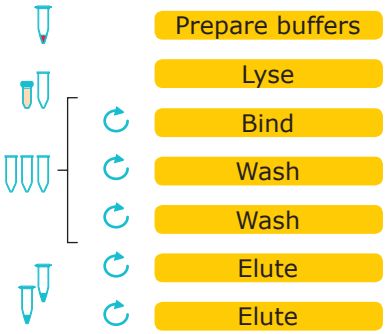
Three key advantages over silica:

- 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.

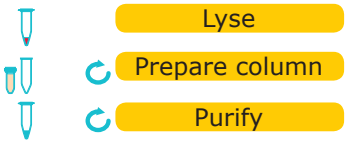
Silica Procedure



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

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.

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.

Yield and Quality of gDNA Isolated from Whole Blood Using GenElute™-E

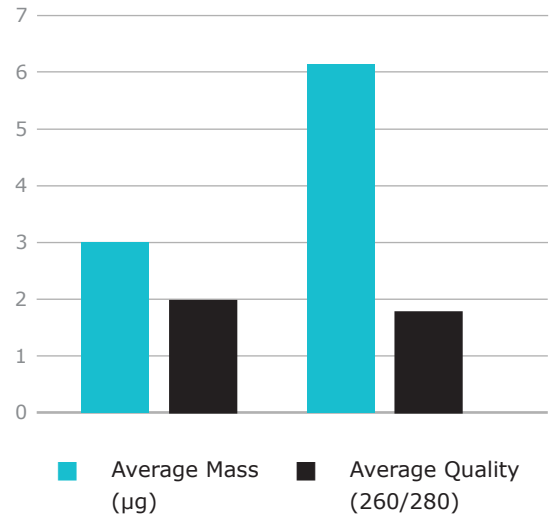


Table 2.

	Average Concentration (ng/µl)	Average Quality (260/280)	Average Mass (µg)
Tilapia Blood	49.87	1.97	4.99
Chicken Blood	61.23	1.82	6.12

Average spectrophotometric results of from forty-eight replicates of gDNA isolation from chicken blood and thirty-two replicates of gDNA isolation from tilapia blood using GenElute™-E Single Spin Blood DNA 96 Kit. Graph shown in Figure 1. Due to sample variability, results may vary.

Storage and Stability

Kit Storage

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.

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

- 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)
  - SmartLyse™ B Protease (P)
  - Clearing Solution B (CS)
  - 1x Tris Buffer (T).

Not Supplied in 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 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:

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

  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 microcentrifuge 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 (T) supplied with the kit.

Troubleshooting

Problem	Probable Cause	Solution
Low yield	Individual samples have inherent variability. In addition, there is variability across different sample types. 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 Purification Plate'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 needs 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.	Additionally, the SmartLyse™ enzymes were optimized to work at the recommended temperature. Verify that the heating unit (ex. thermal shaker) is heating correctly.
	If the preparation steps were performed incorrectly, then the separation resin will be packed incorrectly.	Verify that the preparation steps for the Purification Plate was performed according to the protocol.
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 90-110 µL as that volume is required to displace the Purification Plate buffer.	If the sample volume available to be loaded onto the Purification Plate is below 90 µl, then bring the sample within the recommended range using lysis buffer. If the sample volume available to be loaded onto the Purification Plate is above 110 µl (impacting results), 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 Purification Plate will not impact the ability of the Purification Plate 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.

The purified sample has a reddish color.	In these cases, the Purification Plate may be overloaded due to variations in sample type.	Reduce the amount of sample used with the kit.
	Reddish coloration may indicate incomplete digestion for the sample type.	Try increasing the lysis time.
Purification Plates with dried resin	In rare cases, the Purification Plates dry out during storage. This may be due to not storing the plates according to the recommended conditions.	Store GenElute™-E kits according to the recommended conditions.
Purification Plates with leaks	Although rare, improper sealing or too robust of turbulence can break the seal of the covering of the Purification Plates.	Ensure Purification Plates are efficiently sealed before introducing them to any agitation. If problem persists, reduce the turbulence by reducing the rpm.

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

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Technical Assistance

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

GenElute™-E Single Spin Checklist for Blood DNA 96 Kit

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EC196

Preparation Before Starting

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

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.

Plate Preparation during lysis

Incubate 30 minutes 60 °C, 10 minutes 80 °C

Thermal Shaker 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.

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