User Manual

GenElute™-E Single Spin Tissue DNA 96 Kit

For 96-Well Purification of Genomic DNA from Tissue Samples

EC396

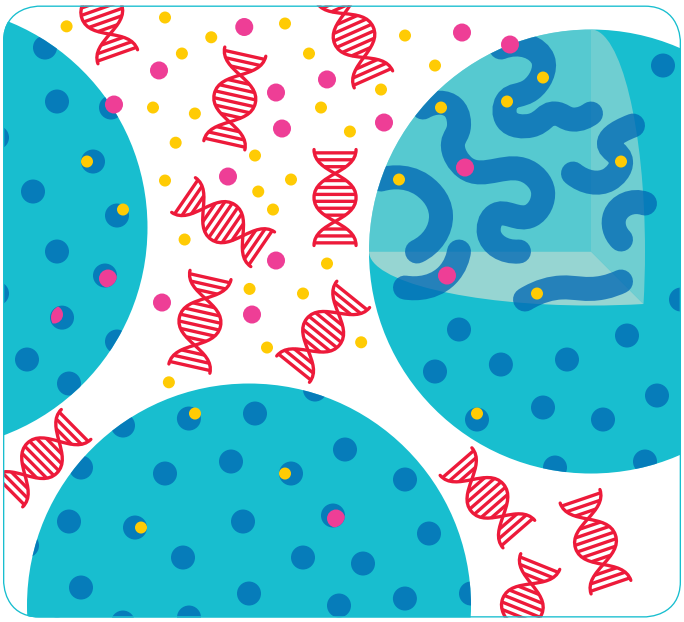
Sigma-Aldrich®

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.

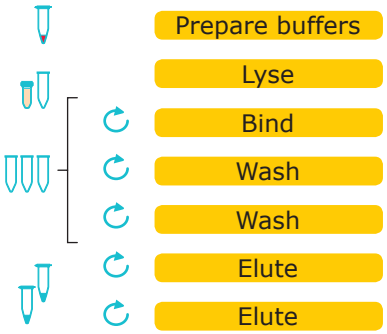
Three key advantages over silica:

- 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.

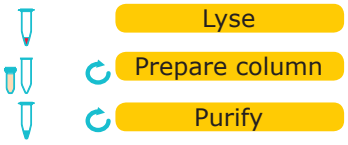
Silica Procedure



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	1-20 mg
Sample Type	Human and animal tissues
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 tissue samples. This protocol has been developed for 1 mg-20 mg human and animal tissue samples. 10 mg is generically recommended (for certain species, optimization of input amount may be required). For high DNA content (e g., spleen, liver, kidney): 5 mg recommended. For low DNA content (e g., muscle, cartilage): 20 mg recommended.

Typical Results

Table 1.

Sample Type	Typical Yield (µg/10mg)
mouse heart	5-10
mouse kidney	5-25
mouse lung	5-20
mouse liver	10-15
mouse brain	2-5
mouse fat	0.5-1.5
mouse muscle	3-5
mouse ear punch	15-30
mouse toe	5-10
rat brain	5-10
rat spleen	5-20
rat muscle	2-10
rat fat	0.1-1
tilapia fins	0.5-1
tilapia gils	0.5-5
tilapia muscle	5-15

Typical yields reflect µg of gDNA per 10mg of initial sample. Due to sample variability, results may vary.

Figure 1.

Yield and Quality of gDNA isolated from Different Mouse Tissue using GenElute™-E

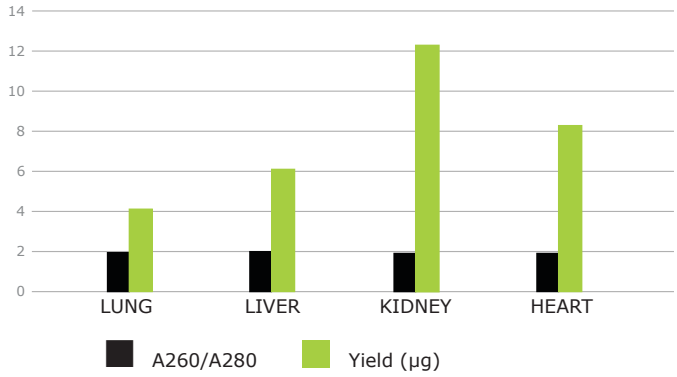


Table 2.

Sample Type	Average Concentration (ng/µl)	Average Quality (260/280)	Average Mass (µg)
Lung	23.55	1.90	4.91
Liver	29.46	2.01	6.17
Kidney	59.51	1.92	12.44
Heart	40.17	1.94	8.39

Average spectrophotometric results of from twenty-four replicates of gDNA isolation from a variety of mouse tissue using GenElute™-E Single Spin Tissue DNA 96 Kit. Graph shown in Figure 1. Due to sample variability, results may vary.

Storage and Stability

Kit Storage

Store SmartLyse™ T Protease (P), RNase A Tissue ® 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 and/or by immediately freezing the sample and storing it at -20ºC or -70ºC can assist in improving results.

However, it is important to keep in mind that individual samples are not homologous during collection and samples often vary between different tissue species as well as among different parts of the tissue itself. Consider optimization of sample homogenization or adjusting the lysis time when working with more difficult sample types.

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 tissue 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 Foils for plate sealing during lysis.
- Reagents:
  - Tissue Lysis Buffer (LB)
  - SmartLyse™ T Protease (P)
  - RNase A Tissue ®
  - Clearing Solution T (CS)
  - 1x Tris Buffer Ⓣ.

Not Supplied with 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 centrifuges

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 pipets for 200 µL scale, corresponding pipet tips.
- Troughs for Master Mix preparation(s) 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. Add 1–20 mg of tissue sample per well of Lysis Plate.

   Note: To avoid degradation, keep samples on ice or in a cooling block during sample loading.

   - If possible, cut tissue into small pieces to speed up lysis.
   - For stabilized tissue samples briefly rinse with water to remove traces of stabilization solution before adding samples to the reaction tube.

2. For individual loading, transfer 130 µL Tissue Lysis Buffer LB and 5 µL SmartLyse™ T Protease P to each well. Otherwise, prepare Lysis Master Mix with 20% excess volume for the number of samples (see table).

Number of samples	1	96 (+20%)
Tissue Lysis Buffer LB	130 µL	14,976 µL
SmartLyse™ T Protease P	5 µL	576 µL
Final Volume	135 µL	15,552 µL

   Add 135 µL of the Lysis Master Mix per well of Lysis Plate.

3. Seal Lysis Plate tightly with Adhesive Foil.

4. 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.

   Note: If samples are not completely lysed after the time period described above, continue with the next step. Residual cellular debris will not interfere with the purification performance.

   Note: For some tissue types, lysis is already complete after 15 minutes. Therefore, this step may be shortened accordingly.

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

5. After incubation at 60 °C, increase the temperature to 80 °C and incubate for additional 10 minutes with maximum agitation.

6. After having performed lysis, detach the Adhesive Foil from the incubated Lysis Plate and add 15 µL Clearing Solution T CS to well of the Lysis Plate. Mix by pipetting up and down. The sample will become cloudy.

   Note: Although RNase digestion is not necessary for most tissue samples, certain tissues contain higher RNA amounts which can be eliminated with an RNase digest. In these cases, prepare a RNase Digest Master Mix with RNase A Tissue ® and Clearing Solution T CS as shown.

Number of samples	1	96 (+20%)
Clearing Solution T CS	15 µL	1,728 µL
RNase A Tissue ®	1 µL	115.2 µL
Final Volume	16 µL	1,843.2 µL

7. Add 16 µL of the RNase Digest Master Mix per well of Lysis Plate. Mix by pipetting up and down and incubate at room temperature for 2 minutes.
8. Centrifuge Lysis Plate for 3 minutes at maximum speed.

Preparation of Purification Plate

9. 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.

10. 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).
11. Place conditioned Purification Plate on top of the Storage Plate for collection of purified DNA.

Purification of DNA

12. Transfer a maximum of 100 µL per well of lysis supernatant containing the DNA into the prepared Purification Plate (maximum of 100 µL per well). 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 sample 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.
13. Centrifuge Purification Plate on top of the Storage Plate for 1 minute at 1,000 x g. The purified DNA flows through the well 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.

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.
	Insufficient sample homogenization or sample shearing can decrease sample yield.	Optimize tissue disruption to minimize shearing of the nucleic acid. Visually inspect that the sample is completely homogenized before using with GenElute™-E kits.
	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.
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.
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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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 Tissue DNA 96 Kit

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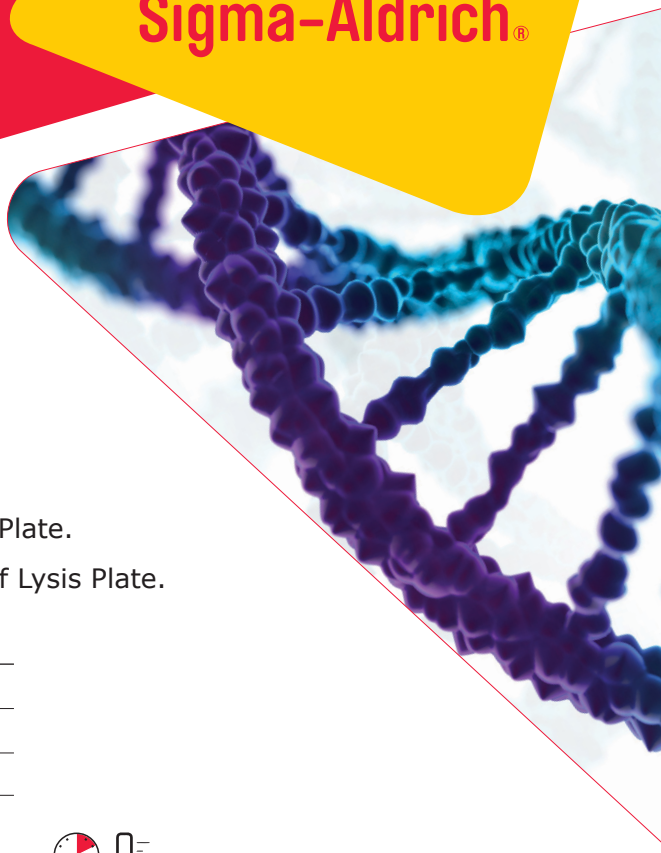

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Preparation Before Starting

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

Lysis

- ☐ Add 1–20 mg of tissue sample per well of Lysis Plate.
- ☐ Prepare Lysis Master Mix, add 135 µL per well of Lysis Plate.

Number of samples	1	96 (+20%)
Tissue Lysis Buffer (LB)	130 µL	14,976 µL
SmartLyse™ T Protease (P)	5 µL	576 µL
Final Volume	135 µL	15,552 µL

Plate Preparation during lysis

Seal Lysis Plate tightly with Adhesive Foil. Incubate 30 minutes 60 °C, 10 minutes 80 °C

Thermal Shaker maximum agitation

- ☐ Remove Adhesive Foil. Prepare RNase Digest Master Mix, add 16 µL per well of Lysis Plate.

Number of samples	1	96 (+20%)
Clearing Solution T (CS)	15 µL	1,728 µL
RNase A Tissue ®	1 µL	115.2 µL
Final Volume	16 µL	1,843.2 µL

- ☐ 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 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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