User Manual

GenElute™-E Single Spin Cleanup Kits

DNA Cleanup Kit EC600

For depletion of impurities and partial fractions (<50 bp) from DNA solutions

Organic Solvent DNA Cleanup Kit EC700

For depletion of impurities and partial fractions from DNA solutions

RNA Cleanup Kit EC800

For elimination of inhibitors, TRIzol™ reagents, organic solvents and a wide range of impurities typically used for isolation from RNA solutions

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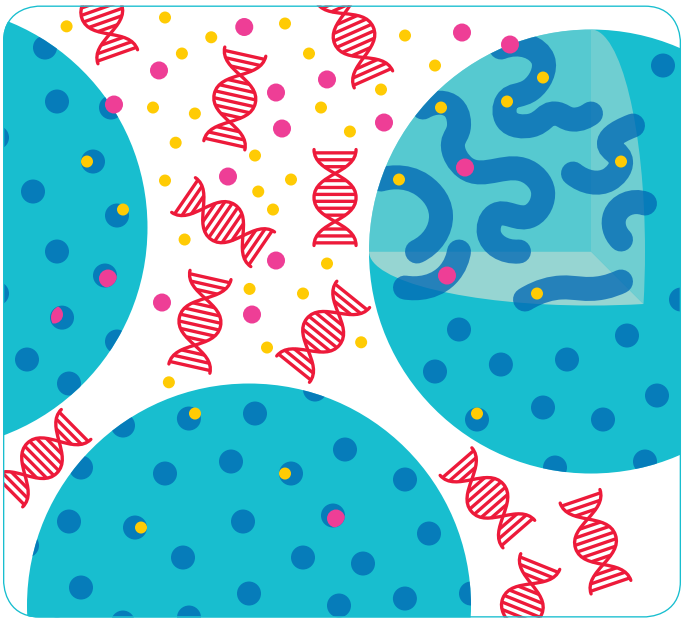
For research use only.

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.

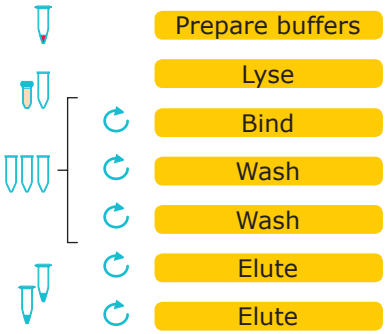
Three key advantages over silica:

- 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.

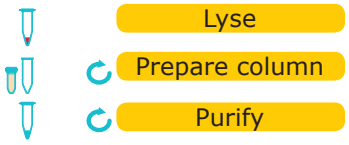
Silica Procedure



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

Selection by Impurity

	Organic solvents				DNA		Salts				Dyes	
	Phenol	TRIzol™ reagent	Chloroform	Ethanol	Primer	dNTPs	Chaotrophs[1]	Salts	SDS	NaAzide	Indigo	Gel loading
GenElute™-E Single Spin DNA CleanUp Kit				✓	✓	✓	✓	✓	✓	✓	✓	✓
GenElute™-E Organic Solvent DNA CleanUp Kit	✓	✓	✓	✓				✓				
GenElute™-E Single Spin RNA CleanUp Kit[2]	✓	✓	✓	✓			✓	✓				

Selection by Application

	Post Phenol-based Extraction[3]	Desalting of Nucleic Acids	Post Bind-Wash-Elute Extraction	PCR CleanUp	Enzymatic Reaction CleanUp	Buffer Exchange[4]
GenElute™-E Single Spin DNA CleanUp Kit		✓	✓	✓	✓	✓
GenElute™-E Organic Solvent DNA CleanUp Kit	✓	✓				✓
GenElute™-E Single Spin RNA CleanUp Kit[2]	✓	✓	✓			✓

1. Gu HCl; GTC
2. Does not recover miRNAs, tRNAs, small RNA molecules
3. TRIzol™ reagents; Phenol/Chloroform based extractions
4. Buffering to low mM Tris, pH 8.2

Intended Use

DNA Cleanup Kit (EC-600)

For single-step purification of DNA. This protocol has been developed to deplete impurities (e. g., salts, nucleotides and DNA fragments or primers of different length (< 50 bp).

Organic Solvent DNA Cleanup Kit (EC-700)

For purification of genomic DNA from DNA solutions. This protocol has been developed to deplete impurities like inhibitors, salts, nucleotides, TRIzol™ reagents, phenol, chloroform and other organic solvents from DNA solutions.

RNA Cleanup Kit (EC-800)

Depletes impurities like inhibitors, salts, nucleotides, TRIzol™ reagents, phenol, chloroform and other organic solvents, typically used for isolation from RNA solutions.

Specifications

Required Time	2 minutes
Purified Nucleic Acid	DNA > 200 or RNA > 30 bp
Final 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

Storage and Stability

Kit Storage

GenElute™-E Cleanup Kits 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.

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

EC600

- DNA Cleanup Spin Columns ◯
- 1x Tris Buffer Ⓣ

EC700

- Organic Solvent DNA Cleanup Spin Columns ◯
- 1x Tris Buffer Ⓣ

EC800

- RNA Cleanup Spin Columns ◯
- 1x Tris Buffer Ⓣ

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.

- Vortex device.
- Pipets for 10 µL, 200 µL, and 1,000 µL scales, corresponding pipet tips.
- One reaction tube (1.5 mL or 2.0 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 nucleic acid.
- GenElute™-E Single Spin Cap Puncher

Preparation before starting

- Set the microcentrifuge to 1,000 x g.

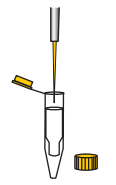
Standard Protocol

Column Preparation

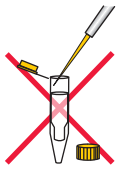
1. Vortex the GenElute™-E Spin Column briefly and place into a 2 mL reaction tube for 5 minutes before continuing.
2. Loosen the screw cap of the Spin Column. Remove from the reaction tube and break off the bottom closure of the column. The screw cap must stay loosened half a turn to avoid generation of a vacuum. Place the Spin Column back into the 2 mL reaction tube.
3. Centrifuge for 1 minute at 1000 x g. Discard the 2 mL reaction tube containing the column buffer.
4. Place the prepared Spin Column into a new 1.5 mL reaction tube for collection of the purified nucleic acid and place back into the rack.

Purification of Nucleic Acid

5. Transfer a maximum of 80-110 µL of sample containing the nucleic acid into the prepared Spin Column:
   - 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 sample, do not touch the resin bed with your pipette tip.
6. Centrifuge for 1 minute at 1000 x g. The purified nucleic acid flows through the column into the 1.5 mL storage tube. Discard the Spin Column.

The collected nucleic acid 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.

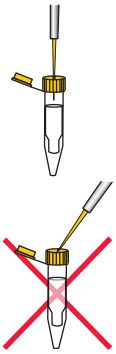
Cap Puncher Protocol

Column Preparation

1. Vortex the GenElute™-E Spin Column briefly and place into a 2 mL reaction tube for 5 minutes before continuing.
2. 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.
3. Centrifuge for 1 minute at 1,000 x g. Discard the 2 mL reaction tube containing the column buffer.
4. Place the prepared Spin Column into a new 1.5 mL reaction tube for collection of the purified nucleic acid and place back into the rack.

Purification of Nucleic Acid

5. Transfer 80 to 100 µl of nucleic acid solution into the prepared Spin Column:
   - 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.
6. Centrifuge for 1 minute at 1,000 x g. The purified nucleic acid flows through the column into the 1.5 mL storage tube. Discard the Spin Column.

The collected nucleic acid 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.supplied with the kit.

Troubleshooting

Problem	Probable Cause	Solution
Low yield	Individual samples have inherent variability. Optimization needs to be performed by the user to validate for their sample type.	Degraded nucleic acid fragments below < 60 bp are depleted during purification.
	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 RT-qPCR Ct values will provide a more reliable measurement of full-length nucleic acid.
	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.
	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.
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.
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.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Standard Warranty

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.

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GenElute™-E Single Spin Checklist for Cleanup Kits

EC600 EC700 EC800

Prepare before starting

- ☐ Set the microcentrifuge to 1000 x g.

Column preparation

- ☐ Vortex Spin Column and place in a 2 mL tube for 5 minutes before continuing.

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 1000 x g to collect column buffer.
- ☐ Place column in a 1.5 mL tube.

Purification of DNA

- ☐ Transfer 80 - 110 µL of sample to column.
- ☐ Centrifuge 1 minute at 1000 x g to collect nucleic acid.
- ☐ Collected nucleic acid is ready to use.

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