ThermoFisher SCIENTIFIC

GeneJET™ RNA Cleanup and Concentration Micro Kit

USER GUIDE

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Revision A

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN1001535 A (English)

Revision	Date	Description
A	1 May 2025	• Initial release with new publication number. Supersedes publication number MAN0012671, Rev. 1. • Updated to the current document template, with associated updates to the limited license information, warranty, trademarks, and logos. • The contents and storage table was updated to include additional instructions for storage of purification columns.

The information in this guide is subject to change without notice.

Contents

1 Product information

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The GeneJET™ RNA Cleanup and Concentration Micro Kit is designed for rapid and efficient concentration of pre-purified RNA samples, as well as for RNA cleanup after DNase I treatment and other enzymatic reactions.

The kit combines the convenience of spin column technology with the selective binding properties of a silica membrane, eliminating the need for tedious resin manipulations or toxic phenol-chloroform extractions.

The standard procedure takes approximately 4 minutes. The purified high-quality RNA can be used in a wide range of downstream applications such as RT-PCR, RT-qPCR, Northern blotting, and other RNA-based analysis.

Technology overview

The GeneJET™ RNA Cleanup and Concentration Micro Kit is based on the ability of RNA to bind to silica membranes in the presence of chaotropic salts, which denature proteins. RNA adsorbs to the silica membrane while contaminants pass through the column. Impurities are subsequently removed from the silica membrane by the addition of Wash Buffer I and Wash Buffer II, and the pure RNA is effectively eluted with water, nuclease-free. The purified RNA is used for a wide variety of downstream applications.

Contents and storage

Table 1 Components of the GeneJET™ RNA Cleanup and Concentration Micro Kit

Component	Cat. No. K0841 (50 reactions)	Cat. No. K0842 (250 reactions)	Storage
Binding Buffer	15 mL	75 mL	15–25°C
Wash Buffer I (concentrated)	2 × 7.5 mL	75 mL	
Wash Buffer II (concentrated)	2 × 7.5 mL	2 × 40 mL	
Water, nuclease-free	30 mL	4 × 1.25 mL 30 mL	
GeneJET™ RNA Purification Micro Column and Collection Tube	50	250	15–25°C For better long-term performance store at 2°C to 8°C.
Collection Tubes, 1.5 mL	50	250	15–25°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Equipment	
Adjustable micropipettors	**MLS**
Multi-channel micropipettors	**MLS**
Vortex mixer, or equivalent	**MLS**
Centrifuge capable of 14,000 × *g* for 1.5 mL Collection Tube	**MLS**
Tubes and other consumables	
RNase-free pipette tips (sterile)	**MLS**
Disposable gloves	**MLS**
Ethanol, 96–100% (molecular biology grade)	**MLS**
DNase I	EN0521
RiboLock RNase Inhibitor	EO0381

Procedural guidelines

- Wear gloves when handling the Binding Buffer and Wash Buffer I as these solutions contain irritants and are harmful if in contact with skin, inhaled, or swallowed.
- All purification steps are performed at room temperature (15–25°C).

Recommendations for avoiding RNA contamination

RNA purity and integrity is important for downstream applications. RNA can be degraded by RNase A, which is a highly stable contaminant found in any laboratory environment. Care must be taken not to introduce RNases into RNA preparation, especially during the column wash and RNA elution steps.

- Wear gloves when handling reagents and RNA samples, as skin is a common source of RNases. Change gloves often.
- Use sterile, disposable RNase-free pipette tips.
- Use appropriate reagents to remove RNase contamination from non-disposable items and work surfaces.
- Keep all kit components tightly sealed when not in use. After usage, close bottles immediately.

Before first use of the kit

1. Add the indicated volume of ethanol (96–100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) before first use.

Component	Cat. No. K0841 (50 reactions)		Cat. No. K0842 (250 reactions)	
Wash Buffer (concentrated)	**Wash Buffer I (2 bottles)**	**Wash Buffer II (2 bottles)**	**Wash Buffer I (1 bottle)**	**Wash Buffer II (2 bottles)**
Wash Buffer (concentrated)	7.5 mL	7.5 mL	75 mL	40 mL
Ethanol (96–100%)	13 mL	30 mL	125 mL	160 mL
Total volume	**20.5 mL**	**37.5 mL**	**200 mL**	**200 mL**

2. Mark the checkbox on the bottlecap to indicate that ethanol has been added to the bottle.

2 Methods

Concentrate RNA

1. Adjust the volume of the reaction mixture to 200 µL with water, nuclease-free.
2. Add 100 µL of Binding Buffer. Mix thoroughly by pipetting.
3. Add 300 µL of ethanol (96-100%), then mix by pipetting.
4. Transfer the mixture to the GeneJET™ RNA Purification Micro Column preassembled with a collection tube.
5. Centrifuge the column at 14,000 × *g* for 30–60 seconds. Discard the flow-through. Place the column back into the collection tube.
6. Add 700 µL of Wash Buffer I supplemented with ethanol to the column, then centrifuge at 14,000 × *g* for 30-60 seconds. Discard the flow-through, then place the purification column back into the collection tube.
7. Add 700 µL of Wash Buffer II supplemented with ethanol to the column, then centrifuge at 14,000 × *g* for 30–60 seconds. Discard the flow-through, then place the purification column back into the collection tube.
8. Repeat step 7.
9. Centrifuge the empty column for an additional 1 minute at 14,000 × *g* to completely remove residual Wash Buffer.

   IMPORTANT! This step is essential to avoid residual ethanol in the purified RNA solution. The presence of ethanol in the RNA sample may inhibit downstream enzymatic reactions.

10. Transfer the column into a clean collection tube, 1.5 mL.
11. Add 10 µL of Water, nuclease-free to the column. Centrifuge at 14,000 × *g* for 1 minute to elute RNA. Discard the purification column.

    Note:
    - Lower elution volume can be used (6–10 µL) to concentrate eluted RNA. An elution volume <10 µL may slightly decrease RNA yield.
    - Double the elution volume or perform two elution cycles when purifying larger amounts of RNA (>5 µg).

Use the purified RNA immediately in downstream applications, store at -20°C until use, or for prolonged storage (more than 1 month) storage at -70°C is recommended.

Remove DNase I

DNA removal is necessary for certain RNA applications that are sensitive to very small amounts of DNA.

- For DNA removal, proceed to step 1
- For DNase I removal, proceed to step 2

1. Add the following reagents to an RNase-free tube. Incubate at 37°C for 30 minutes

Reagent	Amount
RNA	up to 1 µg or up to 45 µL
10X reaction buffer with $MgCl_2$	5 µL
DNase I, RNase-free (1 U/µL)	1 µL (1 u)
Water, nuclease-free	to 50 µL

Note: RiboLock RNase Inhibitor can be added at 1 U/µL to prevent RNA degradation.

2. Add 250 µL of Binding Buffer. Mix thoroughly by pipetting.

Note: Do not exceed 50 µL RNA sample volume. Binding Buffer cannot be scaled up.

3. Proceed to step 3 on page 7.

A Troubleshooting

Observation	Possible cause	Recommended action
Low yield of purified RNA	Ethanol was not added to the mixture of RNA and Binding Solution.	Make sure that ethanol was added to the mixture of RNA and Binding Solution before applying the sample to the purification column.
	Ethanol was not mixed with the RNA and Binding Solution mixture.	Make sure that after the addition of ethanol to the mixture of RNA and Binding Solution sample was briefly mixed by vortexing or pipetting.
	Ethanol was not added to Wash Buffers I and II.	Make sure that ethanol was added to Wash Buffers I and II prior to the first use. Follow instructions for Wash Buffer preparation. See "Before first use of the kit" on page 6.
	Two elution steps need to be done.	Double the elution volume or perform two elution cycles when purifying larger amounts of DNA (>5 µg).
Purified RNA is degraded	RNase contamination.	To avoid RNase contamination wear gloves during the procedure and change gloves frequently. Use sterile, disposable RNase-free pipette tips. Use reagents designed to remove RNase contamination from non-disposable items (pipettes, centrifuges) and work surfaces.
	Purified RNA was not stored properly.	Purified RNA should be used immediately in downstream applications or stored at -20°C for later use. For prolonged storage (more than 1 month) storage at -70°C is recommended.
Inhibition of downstream enzymatic reactions	Purified RNA contains residual salt.	Use the correct order for the Wash Buffers steps. Always wash the purification column with Wash Buffer I first and then proceed with Wash Buffer II.
		The presence of ethanol in the RNA sample may inhibit downstream enzymatic reactions. Ensure that empty column was centrifuged before elution step. See step 9 on page 7.
DNA contamination	RNA contains DNA.	Digest RNA preparation with DNase I, then concentrate RNA using protocol for DNase I removal from reaction mixture. See "Remove DNase I" on page 8.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

C Documentation and support

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.