Thermo
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PRODUCT INFORMATION

Thermo Scientific
GeneJET RNA Cleanup and Concentration Micro Kit

#K0841, #K0842

www.thermoscientific.com/onebio

#__
Lot __
Expiry Date _

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET RNA Cleanup and Concentration Micro Kit is qualified by concentrating RNA transcript following the protocol outlined in the manual. The quality of concentrated RNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified RNA has an A260/280 ratio between 1.9 and 2.2.

Quality authorized by:  Jurgita Zilinskiene

Rev.1. dd

CONTENTS page

COMPONENTS OF THE KIT

GeneJET RNA Cleanup and Concentration Micro Kit	#K0841, 50 preps	#K0842, 250 preps
Binding Buffer	15 mL	75 mL
Wash Buffer 1 (concentrated)	2 × 7.5 mL	75 mL
Wash Buffer 2 (concentrated)	2 × 7.5 mL	2 × 40 mL
Water, nuclease-free	30 mL	4 × 1.25 mL 30 mL
GeneJET RNA Purification Micro Column & Collection Tube	50	250
Collection Tubes, 1.5 mL	50	250

STORAGE

GeneJET™ RNA Cleanup and Concentration Micro Kit can be stored for up to 12 months at room temperature (15-25°C).

DESCRIPTION

GeneJET RNA Cleanup and Concentration Micro Kit is designed for rapid and efficient concentration of prepurified 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.

PRINCIPLE

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 the Wash Buffer 1 and Wash Buffer 2, and the pure RNA is effectively eluted with Water, nuclease-free. The purified RNA is used for a wide variety of downstream applications.

IMPORTANT NOTES

- Add the indicated volume of ethanol (96-100%) to **Wash Buffer 1** (concentrated) and **Wash Buffer 2** (concentrated) prior to the first use:

	#K0841 50 preps		**#K0842 250 preps**	
	Wash Buffer 1 (2 bottles)	**Wash Buffer 2 (2 bottles)**	**Wash Buffer 1 (1 bottle)**	**Wash Buffer 2 (2 bottles)**
Concentrated solution	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

- After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.
- Wear gloves when handling the **Binding Buffer** and **Wash Buffer 1** as these solutions contain irritants (*see* p. 7 for SAFETY INFORMATION) and are harmful if they come into contact with skin, are inhaled or swallowed.
- All purification steps are performed at room temperature (15-25°C).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and sterile RNase-free pipette tips
- Vortex
- Ethanol (96-100%)
- Microcentrifuge
- Disposable gloves

AVOIDING RIBONUCLEASE CONTAMINATION

RNA purity and integrity is essential 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 the RNA preparation, especially during the column wash and RNA elution steps.

General recommendations to avoid RNase contamination include:

- As skin is a common source of RNases, wear gloves when handling reagents and RNA samples. 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.
- Keep all kit components tightly sealed when not in use. After usage, cap bottles immediately.

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PROTOCOLS

A. General RNA concentration protocol.

Step	Procedure
1	Adjust the volume of the reaction mixture to 200 µL with **Water, nuclease-free** (included).
2	Add 100 µL of **Binding Buffer**. Mix thoroughly by pipetting.
3	Add 300 µL of ethanol (96-100%) and mix by pipetting.
4	Transfer the mixture to the **GeneJET RNA Purification Micro Column** preassembled with a collection tube. Centrifuge the column for 30-60 seconds at 14,000 × g. Discard the flow-through. Place the GeneJET RNA Purification Micro Column back into the collection tube.
5	Add 700 µL of **Wash Buffer 1** (supplemented with ethanol, see p. 3) to the GeneJET RNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Add 700 µL of **Wash Buffer 2** (supplemented with ethanol, see p. 3) to the GeneJET RNA Purification Micro Column and centrifuge for 30-60 seconds at 14,000 × g. Discard the flow-through and place the purification column back into the collection tube.
7	Repeat step 6.
8	Centrifuge the empty GeneJET RNA Purification Micro Column for an additional 1 minute at 14,000 × g to completely remove residual Wash Buffer. **Note.** 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.
9	Transfer the GeneJET RNA Purification Micro Column into a clean **Collection Tube tube, 1.5 mL** (included).
10	Add 10 µL of **Water, nuclease-free** (included) to the GeneJET RNA Purification Micro Column. Centrifuge for 1 min at 14,000 × g to elute RNA. **Notes.** • Lower volume of Water, nuclease-free can be used (6-10 µL) in order to concentrate eluted RNA. Please notice that < 10 µL elution volume slightly decreases RNA yield. • Double the elution volume or perform two elution cycles when purifying larger amounts of RNA (> 5 µg).
11	Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20°C or -70°C until use. **Note.** For prolonged storage (more than 1 month), storage at -70°C is recommended.

B. DNase I removal protocol.

Notes.

- DNA removal is necessary for certain RNA applications that are sensitive to very small amounts of DNA.
- For DNA removal please proceed from step 1.
- For DNase I removal please proceed from step 2.

<table>
<tr><th>Step</th><th>Procedure</th></tr>
<tr><td>1</td><td>Removal of DNA from RNA preparations:
Add to a RNase-free tube:<table><tr><td>RNA</td><td>up to 1 µg or up to 45 µL</td></tr><tr><td>10X reaction buffer with $MgCl_2$</td><td>5 µL</td></tr><tr><td>DNase I, RNase-free (#EN0521)</td><td>1 µL (1 u)</td></tr><tr><td>Water nuclease-free (#R0581)</td><td>to 50 µL</td></tr></table>Incubate at 37°C for 30 minutes.
Note. Thermo Scientific RiboLock RNase Inhibitor (#EO0381), typically at 1 u/µL, can be included in the reaction mixture to prevent RNA degradation.</td></tr>
<tr><td>2</td><td>Add 250 µL of Binding Buffer. Mix thoroughly by pipetting.
Note. Do not exceed 50 µL RNA sample volume. Binding Buffer can not be scaled up.</td></tr>
<tr><td>3</td><td>Go to step 3 of the General RNA concentration protocol (p. 4).</td></tr>
</table>

TROUBLESHOOTING

Problem	Possible cause and solution
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 1 and 2.** Make sure that ethanol was added to Wash Buffers 1 and 2 prior to the first use. Follow instructions for Wash Buffer preparation on p. 3. **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 1 first and then proceed with Wash Buffer 2. The presence of ethanol in the RNA sample may inhibit downstream enzymatic reactions. Please ensure that empty GeneJET RNA Purification Micro Column was centrifuged before elution step (see p.4, step 8).
DNA contamination	Digest RNA preparation with DNase I (#EN0521) and concentrate RNA using protocol for DNaseI removal from reaction mixture (*see* p. 5).

Binding Buffer

Xn Harmful

Hazard-determining component of labelling: **Guanidinium thiocyanate.**

Risk phrases

20/21/22	Harmful by inhalation, in contact with skin and if swallowed.
32	Contact with acids liberates very toxic gas.
52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases

9	Keep container in a well-ventilated place.
23	Do not breathe gas/fumes/vapour/spray.
36/37	Wear suitable protective clothing and gloves.
60	This material and its container must be disposed of as hazardous waste.
61	Avoid release to the environment. Refer to special instructions/safety data sheets.

Wash Buffer 1

Xn Harmful

Hazard-determining component of labelling: **Guanidinium hydrochloride.**

Risk phrases

22	Harmful if swallowed.
38	Irritating to skin.
41	Risk of serious damage to eyes.
52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

Safety phrases

S23	Do not breathe gas/fumes/vapour/spray.
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37	Wear suitable protective clothing and gloves.
S60	This material and its container must be disposed of as hazardous waste

Patent pending

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to http://www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.