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PRODUCT INFORMATION

Thermo Scientific
GeneJET Plant RNA Purification Mini Kit
#K0801, #K0802

www.thermoscientific.com/onebio

#K0801, #K0802
Lot __
Exp. __

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Plant RNA Purification Mini Kit is qualified by isolating total RNA from 50 mg of plant tissue following the protocol outlined in the manual. The quality of isolated RNA is evaluated spectrophotometrically and by agarose gel electrophoresis. The purified RNA has an $A_{260/280}$ ratio between 1.9 and 2.1 and the RNA integrity number (RIN) of ≥7.

Quality authorized by: Jurgita Žilinskienė

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COMPONENTS OF THE KIT

GeneJET Plant RNA Purification Mini Kit	#K0801 50 preps	#K0802 250 preps
Plant RNA Lysis Solution	40 mL	200 mL
Wash Buffer WB 1 (concentrated)	40 mL	200 mL
Wash Buffer 2 (concentrated)	30 mL	2 × 100 mL
Water, nuclease-free	30 mL	125 mL
GeneJET RNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes, 2 mL	50	250
Collection Tubes, 1.5 mL	50	250

STORAGE

All components of the kit should be stored at room temperature (15-25°C).
Note. Close the bag with GeneJET RNA Purification Columns tightly after each use!

DESCRIPTION

GeneJET™ Plant RNA Purification Mini Kit is designed for rapid and efficient purification of high quality total RNA from wide variety of plant species and tissue types. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 20 minutes following cell lysis. RNA yields vary between different species, tissues and age of tissue sample. 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. See Table 1 for typical total RNA yields from various sources.

PRINCIPLE

Cells are lysed in a buffer containing guanidine thiocyanate, a chaotropic salt capable of protecting RNA from endogenous RNases. The lysate is then mixed with ethanol and loaded on the purification column. The chaotropic salt and ethanol cause RNA binding to a silica membrane during the lysate is spun through the column. Impurities are effectively removed in subsequent wash steps. Pure RNA is then eluted under low ionic strength conditions with the nuclease-free water provided in the kit.

Table 1. Typical total RNA yields from 50 mg of fresh tissue from various sources.

Plant	Tissue	Yield, µg
Arabidopsis thaliana	leaf	17-20
Nicotiana tabacum	leaf	17-21
Tomato	leaf	50-53
Dandelion	leaf	30-33
Spinach	leaf	25-30
Corn	leaf	25-30
	seeds	6-7
Sugar-cane	leaf	4-5
Rape	leaf	25-30
	roots	20-22
	seeds	40-42
Onion	leaf	10-11
Lettuce	leaf	10-11
Lucerne	leaf	35-40
Cucumber	fruit	40-45
Lemon	leaf	5-7
Rice	leaf	20-25
Wheat	leaf	60-65
Sunflower	stalk	10-11
	seeds	10-12
Sugar-beet	roots	6-7
Soy	seeds	5-7

IMPORTANT NOTES

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

	#K0801 50 preps		**#K0802** 250 preps	
	Wash Buffer WB 1	**Wash Buffer 2**	**Wash Buffer WB 1**	**Wash Buffer 2**
Concentrated wash solution	40 mL	30 mL	200 mL	100 mL
Ethanol (96-100%)	**2.1 mL**	**30 mL**	**10.5 mL**	**100 mL**
Total volume:	42.1 mL	60 mL	210.5 mL	200 mL

After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- Before each RNA purification experiment prepare fresh amount of Plant RNA Lysis solution supplemented with Dithiothreitol (DTT, #R0861). Add 10 µL of 2 M DTT to each 500 µL of **Plant RNA Lysis Solution** used.
- Check **Plant RNA Lysis Solution** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use.
- Wear gloves when handling the **Plant RNA Lysis Solution and Wash Buffer WB 1** as these solutions contain irritants (*see* p.11 for SAFETY INFORMATION) and are harmful if contacted with skin, inhaled or swallowed.
- Perform all purification steps, except the first one, at room temperature (15-25°C).

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipettes and pipette tips
- Ethanol (96-100%)
- DTT, #R0861
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Disposable gloves
- Water bath/incubator 56˚C

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 RNA preparation, especially during the column wash with Wash Buffer 2 and elution steps. General recommendations to avoid RNase contamination:

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

RNA HANDLING AND STORAGE

- Keep the RNA on ice after extraction and while working with it.
- Store the extracted RNA at -20°C or -70°C. For long term stability, keep the RNA at -70°C.

PROTOCOLS

Protocols for RNA purification from lignified, polyphenol-rich plant tissues and soybean seeds are described on p.7.

Before starting: Supplement the Plant RNA Lysis Solution with DTT (#R0861). Add 10 µL of 2 M DTT to each 500 µL of Plant RNA Lysis Solution required for the extraction procedure.

A. Plant RNA Purification Main Protocol

Step	Procedure
1	Pipette 500 µL of **Plant RNA Lysis Solution,** supplemented with DTT, into 1.5 mL microcentrifuge tube (not provided). Weigh the plant tissue - use up to 100 mg of fresh or frozen tissue; up to 20 mg of lyophilized tissue. Grind the material using one of the following methods: a) Mortar and Pestle Place up to 100 mg of plant tissue into liquid nitrogen and grind thoroughly with a mortar and pestle. b) Grinding mill Place up to 100 mg of tissue into a vial containing stainless steel beads. The vial and beads should be precooled with liquid nitrogen. The setup of the mechanical disruption depends on the tissue type. Immediately transfer the tissue powder into a 1.5 mL microcentrifuge tube containing 500 µL of Plant RNA Lysis Solution. Vortex for 10-20 s to mix thoroughly. ***Note*** • *Transfer the ground tissue to the Plant RNA Lysis Solution as quickly as possible to avoid RNA degradation.* • *All ground material must be thoroughly mixed with the Plant RNA Lysis Solution. RNA degradation can occur in particles that are left to dry on the walls of the tube.* • *Ground tissue can be used immediately in the RNA isolation protocol or stored at -70°C until use.*
2	Incubate for 3 min at 56°C. Centrifuge for 5 min at ≥20,000 × g (≥14,000 rpm).
3	Collect the supernatant (usually 450-550 µL) and transfer to the clean microcentrifuge tube (not provided). Add 250 µL of 96% ethanol. Mix by pipetting.
4	Transfer the prepared mixture to a purification column inserted in a collection tube. Centrifuge the column for 1 min at 12,000 × g (~11,000 rpm). Discard the flow-through solution and reassemble column and collection tube. ***Note.*** *Close the bag with GeneJET RNA Purification Columns tightly after each use!*
5	Add 700 µL of Wash Buffer WB 1 to the purification column (ensure ethanol has been added to Wash Buffer WB 1). Centrifuge for 1 min at 12,000 × g (~11,000 rpm). Discard the flow-through and collection tube. Place the purification column into a clean 2 mL collection tube.

6	Add 500 µL of Wash Buffer 2 to the purification column (ensure ethanol has been added to Wash Buffer 2). Centrifuge for 1 min at 12,000 × g (~11,000 rpm). Discard the flow-through solution and reassemble column and collection tube.
7	Repeat Step 6 and re-spin the column for 1 min at maximum speed ≥20,000 × g (≥14,000 rpm). Discard the collection tube containing the flow-through solution and transfer the purification column to a RNase-free 1.5 mL collection tube.
8	To elute the RNA, add 50 µL of nuclease-free water to the centre of the purification column membrane and centrifuge for 1 min at 12,000 × g (~11,000 rpm). ***Note**: Repeat the elution step when yields greater than 30 µg are expected.*
9	Discard the purification column. Use the purified RNA immediately in downstream applications or store at -20°C until use. Keep the RNA on ice after extraction and while working with it. ***Note.** For prolonged storage (more than 1 month) storage at -70°C is recommended.*

B. RNA Purification from Lignified, Polyphenol-rich Plant Tissues

To purify RNA from woody, lignified and/or polyphenol-rich samples such as branches, twigs, needles, wax-coated leaves (e.g. laurel, vine) and wheat flour, supplement the lysis mix with polyvinylpyrrolidone (PVP).

Step	Procedure
1	In a 1.5 mL microcentrifuge tube (not provided) add 500 µL of **Plant RNA Lysis Solution** supplemented with DTT (as described in p.5) and PVP at a 2% (w/v) final concentration. Grind up to 100 mg of plant material in liquid nitrogen using a mortar and pestle or grinding mill as described in Step 1 on p.5. Transfer the tissue powder immediately into a 1.5 mL microcentrifuge tube containing 500 µL of Plant RNA Lysis Solution, supplemented with DTT and PVP. Vortex for 10-20 s to mix thoroughly. *Optional: for tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 min.*
2	Incubate for 5 min at 56°C. Centrifuge for 5 min at ≥20,000 × g (≥14,000rpm).
3	Proceed to step 3 of the Plant RNA Purification Main Protocol on p.5.

C. RNA Purification from Soybean seeds

To purify of RNA soy beans it is necessary to supplement the lysis mix with Sodium Chloride.

Step	Procedure
1	In a 1.5 mL microcentrifuge tube (not provided) add 500 µL of **Plant RNA Lysis Solution** supplemented with DTT (as described in p.5) and NaCl at a 2 M final concentration. Grind up to 100 mg of plant material in liquid nitrogen using a mortar and pestle or grinding mill as described in Step 1 on p.5. Transfer the tissue powder immediately into a 1.5 mL microcentrifuge tube containing 500 µL of Plant RNA Lysis Solution, supplemented with DTT and NaCl. Vortex for 10-20 s to mix thoroughly. *Optional: for tissues resistant to mechanical disruption, add glass sand to the microcentrifuge tube and vortex for 1 min.*
2	Proceed to step 2 of the Plant RNA Purification Main Protocol on p.5.

D. Genomic DNA Removal from RNA Preparations

1. Using DNase I, stand-alone product (#EN0521)

Step	Procedure
1	Add to an RNase-free tube: RNA 1 µg 10X reaction buffer with $MgCl_2$ 1 µL DNase I, RNase-free (#EN0521) 1 µL (1 u) Water, nuclease-free (#R0581) to 10 µL Incubate at 37°C for 30 min. ***Note.*** *To prevent RNA degradation, Thermo Scientific RiboLock RNase Inhibitor (#EO0381), can be added to the reaction mixture at a final concentration of 1 u/µL.*
2	Add 1 µL 50 mM EDTA and incubate at 65°C for 10 min to inactivate the DNase I. Addition of EDTA is required as RNA hydrolyzes during heating with divalent cations in the absence of a chelating agent.
3	Use the prepared RNA directly for downstream applications.

Note

- Do not use more than 1 u of DNase I per 1 µg of RNA.
- Volumes of the reaction mixture and 50 mM EDTA solution can be scaled up for larger amounts of RNA. The recommended final concentration of RNA is 0.1 µg/µL.
- When working with diluted RNA samples, reaction volumes can be scaled up to accommodate the starting amount of RNA. However, it is important to maintain the 1 u DNase/µg RNA ratio and ~5 mM final EDTA concentration.

2. Using Thermo Scientific RapiOut DNA Removal Kit (#K2981).

The kit is designed for convenient removal of gDNA from RNA sample and subsequent removal of DNase I in a simple two-step procedure. Kit contains recombinant DNase I, RNase-free and a proprietary DNase Removal Reagent for efficient DNase I removal.
For more detailed information, please visit www.thermoscientific.com/onebio.

TROUBLESHOOTING

Problem	Possible cause and solution
Low RNA yield	**Insufficient homogenization of plant material.** To disrupt the cell wall, it is important to homogenize the sample thoroughly until it is ground to fine powder. **Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more than 100 mg of plant tissue per column. **Ethanol was not added to the lysate.** Ensure ethanol was added to the lysate before applying the sample to the purification column. **Ethanol was not mixed properly with the lysate.** After the addition of ethanol to the lysate, mix the sample by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Ensure ethanol was added to Wash Buffer WB 1 and Wash Buffer 2 before use. Follow the instructions for Wash Buffer preparation on p.3. **DTT was not added to Plant RNA Lysis Solution.** DTT should be added fresh to an aliquot of the Plant RNA Lysis Solution prior to the purification procedure. **Sample may be old or degraded** If possible use young leaves or tissues.
Purified RNA is degraded	**RNase contamination.** To avoid ribonuclease contamination, wear gloves during every procedure involving RNA extraction, purification and subsequent applications. Change gloves frequently. Use sterile, disposable RNase-free pipette tips. Treat nondisposable items and work surfaces with solutions designed to eliminate RNases. **Inappropriate sample storage conditions.** Flash-freeze and homogenize plant sample in liquid nitrogen. Immediately transfer homogenized powder into Plant RNA Lysis Solution. Purified RNA should be used immediately in the downstream applications or stored at -20°C for later use. For prolonged storage (more than 1 month) freezing at -70°C is recommended.
Inhibition of downstream enzymatic reactions	**Purified RNA contains residual ethanol.** If residual solution is seen in the purification column after washing the column with Wash Buffer 2, empty the collection tube and re-spin the column for an additional 1 min. at maximum speed (≥20,000 × g, 11,000 rpm). **Purified RNA contains residual salt.** Use the correct order for the Wash Buffers. Always wash the purification column with Wash Buffer WB 1 first and then proceed to washing with Wash Buffer 2.

Problem	Possible cause and solution
Column clogging	**Clarified supernatant contaminated with cell debris.** Make sure not to transfer any pelleted precipitate from the lysate clarifying centrifugation step onto spin column. **To low temperature during centrifugation.** At low temperatures nucleic acids can precipitate and clog the column. Maintain 20-25°C temperature during centrifugation steps.
DNA contamination	**DNA rich plant sample.** The amount of gDNA co-purified with the sample RNA varies with the plant species used and can be substantial. To completely remove gDNA digest the RNA preparation with DNase I (#EN0521) as outlined on p.8.

Plant RNA Lysis Solution

Xn Harmful

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

Risk phrases

R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.

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.

Wash Buffer WB 1

Xn Harmful

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

Risk phrases

R22 Harmful if swallowed.
R36/38 Irritating to eyes and skin.

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

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 www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.