DANAGENE PLANT RNA KIT

REF.0802.1 100 PREPS
REF.0802.2 500 PREPS

1.INTRODUCTION

This kit provides a method for an efficient and fast **total RNA from different cells and tissues of plants and fungi samples using MiniSpin columns.**

The samples are ground under liquid nitrogen followed by incubation in the lysis solution which immediately inactives the RNases and creates the correct binding conditions for the RNA absorption on the silica membrane. Together with the lysis solution, **a PVP (polyvinylpyrrolidone) solution** is added, that acts binding contaminants such us polyssacharides and polyphenols which may interfere or degrade the RNA.
Salt metabolites and cell components are removed by washing with 2 different buffers. The total RNA is eluted with nuclease free-water.

DANAGENE PLANT RNA Kit contains 2 different RNA lysis solutions, one based on guanidine thiocyanate, RNA Lysis Buffer 1, the most recommended due to its high denaturation property, and other based on guanidine HCl, RNA Lysis Buffer 2, as in some plants and fungi the presence of certain metabolites produces a solidification of the lysate.

Features:

- **High-quality total RNA in 30 minutes.**
- **Two alternatives lysis buffers included-optimized lysis procedure.**
- **Sample material: up to 100 mg (fresh plant tissue), up to 25 mg (dry plant tissue).**
- **Elution volume: 30-60 µl.**
- **No phenol/chloroform extraction, no CsCl gradient, no LiCl or etanol precipitation.**

2. KIT COMPONENTS

	100 preps	*500 preps*	*Storage*
RNA Lysis Buffer 1	**45 ml**	**2 x 100 ml**	Room temperature
RNA Lysis Buffer 2	**45 ml**	**2 x 100 ml**	Room temperature
RNA Precipitación Buffer	**4 ml**	**20 ml**	Room temperature
PVP Solution	**4.5 ml**	**22 ml**	4ºC
RNA Wash Buffer 1	**10 ml**	**50 ml**	Room temperature
RNA Wash Buffer 2*	**20 ml**	**4 x 20 ml**	Room temperature
Nuclease-Free water	**8 ml**	**40 ml**	Room temperature
gDNA Removal Column	**100 units**	**500 units**	Room temperature
RNA Column	**100 units**	**500 units**	Room temperature
Collection Tubes	**200 units**	**1000 units**	Room temperature

(*) These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol.

PRECAUTIONS: The RNA Lysis Buffer and RNA Wash solution 1 contains guanidium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined bleach.

Equipment and additional reagents required

- 100 % Ethanol.
- Microcentrifuge.
- Micropipettes.
- RNase-free 1.5 ml or 2.0 ml microfuge tubes.
- Mechanical homogenizer.
- Liquid Nitrogen , mostar and pestle.
- β-mercaptoetanol.

3. PROTOCOL

3.1 Preliminary Preparations

- Add **40 ml Ethanol 100 %** (kit 100 preps) **to RNA Wash Buffer 2** and **200 ml** (kit 500 preps). Keep the container closed to avoid the ethanol evaporation.

3.2 General Remarks

Stabilization of RNA in biological samples

The RNA is not protected until the sample material freezes instantaneously or breaks / lyses in the presence of inhibitors or denaturing agents of the RNases.
Immediate stabilization of the DNA expression pattern is a prerequisite for accurate gene-expression analysis.

Disruption and homogenization of starting materials for RNA isolation

Efficient disruption and homogenization of the starting material is an absolute requirement for all total RNA isolation procedures. Disruption and homogenization are two distinct steps.

Disruption: Complete disruption of cells walls and plasma membranes of cells and organelles is absolutely required to release all the RNA contained in the sample. Different samples requires different methods to archieve complete disruption. Incomplete disruption results in significantly reduced yields.

Homogenization: Is necessary to reduce the viscosity of the cells lysates produced by disruption. Homogenization shears the high-molecular-weight genomic DNA and other high-molecular-weight cellular components to create a homogeneous lysate. Incomplete homogenization results in efficient binding of RNA and therefore significally reduced yields.

Some disruption methods simultaneously homogenize the sample (rotor-stator-homogenizer) while others require an additional homogenization step

3.3 Protocol for RNA purification from plant or fungi tissues

Process samples of up to **25 mg of dry tissue** or **100 mg of fresh tissue** . Grind the sample to a fine powder in the presence of liquid Nitrogen with a pestle and mortar.

IMPORTANT:

- It is necessary for an efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption with a manual mechanical homogenizer (Ejem. Polytron) and that the viscosity of the sample is reduced by homogenization, be careful to keep the rotor submerged to avoid forming too much foam and choose a homogenizer with a 5-7 mm rotor that can be used in microtubes.

- In case of **fungi,** collect the micellium from the culture by filtration, wash 3 times with deionized sterile water or PBS to remove the culture medium. Grind the sample a fine powder with liquid nitrogen, store at –80ºC or process the sample.

- The use of b-mercaptoethanol in lysis is highly recommended for most plant tissues, particularly those know to have a high RNAse content. It is also recommended for users who whish to isolate RNA for sensitive downstream applications. Alternatively, the RNA Lysis Buffer can be used as supplied.

1. Add **400 μl of RNA Lysis Buffer + 40 μl PVP Solution** + **4.0 μl of β-mercaptoetanol** to the ground tissue with liquid Nitrogen. Homogenize with manual electric homogenizer or shear this tissue samples by passing lysate through a 20-G (0.9 mm) needle syringe 10 times. Incubate a room temperature for 5 minutes.

2. Add **30 μl of RNA Precipitation Buffer.** Vortex and incubate for 1 minute.

3. **Centrifuge at maximum speed for 3 minutes.**

4. **Transfer the supernatant to the gDNA Removal Column** and centrifuge for 1 minute at 8.000 rpm.

This step eliminates much of the contaminating genomic DNA, not being total for those applications that require a total elimination, to do this a DNase I treatment on the column or once the RNA is eluted.

5. Discard the column and **continue with the flow-through.**

Note: Ensure that no liquid remains on the column membrane after centrifugation. If necessary, repeat the centrifugation until all liquid has passed through the membrane. If (in rare cases) the flow-through contains obvious undissolved sediment, recover flow-through withoutsediment. Optimize mechanical sample disruption for subsequent preparations.

6. Add **350 μl of Ethanol 100% to the lysate of the point 5.** Mix well.

7. Take a **RNA column** and its collection tube and **add the lysate**. Centrifuge for 1 minute at 8.000-10.000 rpm.

8. Add **100 μl of RNA Wash Buffer 1.** Centrifuge at maximum speed for 1 minute.

9. Add **700 μl of RNA Wash Buffer 2.** Centrifuge at maximum speed for 1 minute.

10. Centrifuge for **3 minutes** at **maximum speed (11.000 x g)** to completely dry the membrane. Place the column in a 1.5 ml (not supplied) microtube.

11. Add **30 μl of Nuclease-Free water.** Incubate for 2 minutes and centrifuge **at maximum speed for 1 minute.**

12. Add again **30 μl of Nuclease-Free water.** Incubate for 2 minutes and centrifuge **at maximum speed for 1 minute.**

4. APPENDIX

DNase digestion in solution

The passage of the lysed sample through gDNA Removal Column according to the standard protocol is very efficient in DNA binding resulting in minimal residual DNA in the purified RNA. Residual DNA will not be detectable in most downstream applications. Despite this, there are still certain applications which require even lower contents of residual DNA. However, removal of DNA to a completely undetectable level is challenging and the efficiency of the gDNA Removal Column is sometimes not sufficient for downstream applications requiring lowest residual content of DNA. This can be especially the case if a large amount of sample or a sample containing much DNA is processed with the gDNA Removal Column.
The amount of residual DNA detected depends on sample type, amount and its DNA content and the detection sensitivity of the method used to analyse residual DNA.

DNA digestion in solution can efficiently destroy contaminating DNA. However, stringent RNase control and subsequent repurification of the RNA (in order to remove buffer, salts, DNase and digested DNA) are usually required.

We recommend the use of our **DANAGENE DNA Removal Kit (ref.0807)** that provides a method to eliminate contaminating genomic DNA in RNA preparations using 2 sequential filtrations with different columns. It should be taken into account that this method reduces the amount of RNA, which is why it is important to decide if it is necessary to completely eliminate the possible contaminating DNA for the realization of our subsequent application.

5. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es