DANAGENE TISSUE/CELLS RNA KIT

Ref. 0801.1 100 preps
Ref. 0801.2 500 preps

1. INTRODUCTION

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

The DANAGENE TISSUE/CELLS RNA Kit integrates a gDNA Removal Column. This Mini spin column removes quickly and efficiently the most genomic DNA without the need of DNase digestion.
In the first step cells and tissues are lysed without the need of β-mercapthoethanol The chaotropic salt included in the lysis buffer immediately inactivates RNases. The lysate is added to the gDNA Removal Column to clarify the lysate and to remove contaminating gDNA. After addition of the binding solution to the flow-through, the RNA is bound to the RNA Column. Afterwards, two washing steps remove salts, metabolites, and macromolecular cellular components. High quality RNA is eluted with RNase-free H2O.

Features:

- **Fast procedure delivering high-quality total RNA in minutes.**
- **Convenient handling – lysate clearing and gDNA removal with one column in one step.**
- **Sample Material: < 1 x 10^7 cultured cells; 25 mg animal/human tissue.**
- **No phenol/chloroform extraction, no CsCl gradients, no LiCl or ethanol precipitation.**

Applications:

- **RNA isolation from cultured cells and animal tissues.**
- **RNA is ready for downstream applications such as RT-PCR, Northern Blotting, Primer Extension, mRNA Selection, cDNA Synthesis, RNase Protection Assay.**

2. KIT COMPONENTS

	100 preps	500 preps
RNA Lysis Buffer	**45 ml**	**2 x 100 ml**
RNA Precipitation Buffer	**4 ml**	**20 ml**
RNA Wash Buffer 1	**10 ml**	**50 ml**
RNA Was Buffer 2*	**20 ml**	**4 x 20 ml**
Nuclease-Free water	**8 ml**	**40 ml**
gDNA Removal Column	**100 units**	**500 units**
RNA Column	**100 units**	**500 units**
Collection Tubes	**200 units**	**1000 units**

(*) 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 guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined bleach.

Equipment and additional reagents required

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

3. PROTOCOL

3.1 Preliminary Preparations

- Add **80 ml Ethanol 100 %** (kit 100 preps) **to RNA Wash Buffer 2** and **80 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.

Methods for sample collection

• Use a freshly collected sample for immediate lysis and RNA purification.

• Samples can be stored in the lysis buffer after lysis at -80 ° C for one year, at 4º C for up to 24 hours or up to several hours at room temperature. Frozen samples in lysis buffer should be thawed slowly before starting RNA isolation.

• Freeze the sample in liquid N2 immediately after collection and store at 80ºC. This can be done with a mortar and pestle. Make sure that the sample does not thaw before contact with the lysis buffer.

• Samples can be submerged and stored in **DANAPROTECT SOLUTION or RNAlater**. Before using such samples, remove excess DANAPROTECT SOLUTION from the tissue before use.

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 human or animal tissues

Process samples of up to **25 mg** of fresh or frozen 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.

For fresh and soft tissues use the homogenizer; for fresh and hard tissues or RNase-rich tissues grind the sample to a fine powder with liquid Nitrogen. For frozen soft tissues use the homogenizer; for the rest of tissues grind the sample to a fine powder with liquid Nitrogen.

RNA isolation from skeletal muscle, heart, and skin tissue can be difficult due to the abundance of contractile proteins, connective tissue, and collagen. In order to remove these proteins, which can interfere with RNA isolation, the sample needs to be created with a protease. However, the protease digest needs to be carried out under conditions that do not allow RNA degradation.

1. Add **400 µl of RNA Lysis Buffer** 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 3 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.**

3.4 Protocol for RNA purification from cells(< 1 x 10^7)

Adherent Cultured Animal Cells
Aspirate cell-culture medium and add an equal amount of PBS in order to wash the cells. Aspirate PBS. Add 0.1-0.3% trypsin in PBS and incubate for an appropriate time to detach the cells the dish surface. After cell detachment , add cell culture medium, transfer cells to an appropriate tube and pellet by centrifugation. Remove supernatant and continue with the point 1.

Suspension Cultured Animal Cells
Transfer cells (up to 1 x 10^7) to a 1.5 ml microcentrifuge tube. . Harvest cells by centrifugation, remove the supernatant and continue with the point 1.

1. Add **400 µl of RNA Lysis Buffer.** Resuspend the cells with the micropipette. Incubate for 5 minutes at room temperature.

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