Precellys Tissue Total RNA Extraction Kit

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Precellys Tissue Total RNA Extraction Kit #D05711

For research laboratory use only

User Manual #D11711

Version: 0121

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Introduction and Overview

The Precellys Tissue Total RNA Extraction Kit provides an easy and rapid method for the isolation of total RNA for subsequent RNA analysis such as RT-PCR, Northern analysis, Poly A+ RNA (mRNA) purification, nuclease protection, and *in vitro* translation. This kit can purify up to 100 µg total RNA from cultured eukaryotic cells or tissue. Normally, 1 x 10^6 - 1 x 10^7 eukaryotic cells, or 5-30 mg tissue, can be processed in a single experiment. Fresh, frozen, or RNA*Later*® stabilized tissues can be used. Cell or tissue lysis occurs under denaturing conditions which inactivate RNases. After the homogenization process, samples are applied to the RNA Mini Column which binds total RNA. Cellular debris and other contaminants are effectively washed away after a few quick wash steps. High-quality RNA is eluted in Nuclease-free Water. Total RNA greater than 200 nt is isolated using this kit.

Binding capacity

Each RNA Mini Column can bind approximately 100 µg RNA. Using greater than 30 mg tissue or 1 x 10^7 cells is not recommended.

Description of protocols

Different protocols in this handbook provide detailed instructions to use the Precellys Tissue Total RNA Extraction Kit for purification of total RNA.

The protocol "**Animal Tissue Protocol**" is for use with the

Precellys Tissue Total RNA Extraction Kit for purification of RNA from animal tissues.

The protocol "**Cultured Cell Protocol**" is for use with the Precellys Tissue Total RNA Extraction Kit for purification of RNA from cultured animal or human cells.

All protocols can be realized by using the "**Vacuum Protocol**".

Kit Contents

The Precellys Tissue Total RNA Extraction Kit is available in 2 sizes:

- D05711.5 ea for 5 extraction prep
- D05711.50 ea for 50 extraction prep

Designation	Quantity per kit	
	D05711.5 ea	D05711.50 ea
RNA Mini Columns	5	50
2 mL Collection Tubes	10	100
RNA Lysis Buffer	5 mL	40 mL
Wash Buffer I	5 mL	50 mL
Wash Buffer II	5 mL	12 mL
Nuclease-free Water	2 mL	30 mL
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Storage and Stability

All Precellys Tissue Total RNA Extraction Kit components are guaranteed until the expiry date stated in the package, when stored at room temperature. During shipment, crystals or precipitation may form in the RNA Lysis Buffer. Dissolve by warming buffer at 37°C.

Precaution for use

Users are recommended to carefully read all instructions for use before starting work.

Please take a few minutes to read this booklet in its entirety to become familiar with the procedures. Prepare all materials required before starting to minimize RNA degradation.

- Whenever working with RNA, always wear gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents
- Equilibrate samples and reagents to room temperature before beginning this protocol. All steps should be carried out at room temperature unless otherwise noted. Work quickly, but carefully.
- Prepare all materials required before starting the procedure to minimize RNA degradation.
- Carefully apply the sample or solution to the center of the RNA Mini Columns. Avoid touching the membrane with pipet tips.
- Optional: 2-mercaptoethanol is key in denaturing

RNases and can be added to an aliquot of RNA Lysis Buffer before use. Add 20 µL 2-mercaptoethanol per 1 mL RNA Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

Handling starting material

It is crucial to protect RNA from degradation by RNases in the samples and during extraction process. We recommend to use at least one of the following protective measure immediately after sample harvesting: flash-freezing the samples, use of RNAse inhibiting reagents or treatment with RNA*later*® RNA stabilization reagent.

Samples should be stored at -70°C or below until RNA extraction procedure, in cryotubes compatible with this temperature range. Please note that Precellys lysing kit tubes are not compatible with temperatures below 0°C.

Preparing Reagents

Dilute **Wash Buffer II** with 100% ethanol as follows and store at room temperature.

Kit	100% ethanol to be added
D05711.5 ea	20 mL
D05711.50 ea	48 ml

Optional: As a preparation step add 20 µL 2-mercaptoethanol (β-mercaptoethanol) per 1 mL RNA Lysis Buffer. This mixture can be stored for 4 weeks at room temperature.

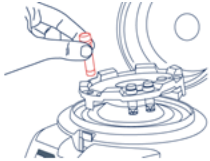
Recommended Precellys protocols

Species	Sample type	Precellys lysing kit	Precellys Evolution Speed (rpm)	Precellys 24 Speed (rpm)	Recommended program
Mouse	Heart	CK28R 2 mL	8500	6500	3 cycles of 10 s 30 s break between cycles
Mouse	Liver	CK14 2mL	8000	6000	1 cycle of 10 s No break
Mouse	Brain	CK14 2mL	8000	6000	1 cycle of 15 s No break
Mouse	Lung	CK28R 2 mL	8500	6500	3 cycles of 15 s 15 s break between cycles
Mouse	Muscle	CK28R 2 mL	8500	6500	3 to 6 cycles of 20 s 15 s break between cycles

Rabbit	Eye	CK14 2mL	8200	6500	3 cycles of 30 s 30 s break between cycles
Mouse	Cartilage tumor	CK28R 2 mL	8500	6500	3 cycles of 15 s 10 s break between cycles
Mouse	Bone	MK28R 2 mL	8500	6500	6 to 8 cycles of 10 s 15 s break between cycles
Mouse	Tail clip	CKMix	8500	6500	3 cycles of 20 s 15 s break between cycles
-	Cultured cells	CK14	7500	5000	2 cycles of 20 s 15 s break between cycles

Note: RNA yield and integrity can be optimized by using a Precellys Evolution combined to Cryolys Evolution.

Illustrated protocol



Sample homogenization and lysis

Add ethanol

Apply sample to column

Wash 3X

Dry

Elute

Materials and equipment required to be supplied by User

In addition to standard laboratory equipment, the following materials are required:

- Precellys tubes adapted to the type of samples targeted (see section «Recommended Precellys protocols»)
- Tabletop microcentrifuge capable of 14,000 x g
- RNAse-free pipette tips and 1.5 mL microcentrifuge tubes
- 100% ethanol
- 70% ethanol in sterile DEPC-treated water
- Optional: 14.3M 2-mercaptoethanol (β-mercaptoethanol)
- Vacuum Manifold and Vacuum source (for Vacuum protocol only)

Before Starting

Before starting any following protocols:

- Prepare Wash Buffer II according to the "Preparing Reagents" section.
- Optional: add 20µL 2-mercaptoethanol per 1 mL RNA Lysis Buffer

Animal Tissue Protocol

1. Determine the proper amount of starting material

Note: It is critical to use the correct amount of tissue in order to obtain optimal yield and purity with the RNA Mini Column. The maximum amount of tissue that can be processed with the Total RNA Protocol is dependent on the tissue type and its RNA content. The maximum binding capacity of the RNA Mini Column is 100 µg. The maximum amount of tissue that RNA Lysis Buffer can lyse in this protocol is 30 mg. Use the table below as a guideline to select the correct amount of starting material. If no information regarding your starting material is available, begin with 10 mg. Based on RNA yield and quality obtained from 10 mg, the starting amount can be adjusted for the next purification.

Source	Amount of Tissue (mg)	RNA Yield (µg)
Brain	10	10
Kidney	10	30
Liver	10	45
Heart	10	5
Spleen	10	33
Lung	10	12
Pancreas	10	40
Thymus	10	20

Average Yield of Total Cellular RNA from Mouse Tissue

2. Add the proper amount of tissue in the recommended Precellys lysing kit tube (see section «Recommended Precellys protocols»). For optimal tissue disruption, cut

the sample into small pieces of approximatively 2 mm^3 before transfer.

Note: For samples stored in RNA*Later*®:

- Spin down the tissue at 500 x g for 5 minutes.
- Aspirate out the supernatant.
- Transfer the pelleted tissue in the recommended Precellys tube

3. Add the correct amount of RNA Lysis Buffer based on the recommendation below:

Amount of Tissue	Volume of RNA Lysis Buffer (µL)
≤ 15 mg	350 µL
20-30 mg	700 µL
samples stored in RNA*Later*®	700 µL

Amount of RNA Lysis Buffer per Tissue Sample

4. Use the recommended Precellys protocol to disrupt the tissue based on the nature of the sample.

5. Centrifuge at maximum speed for 5 minutes.

6. Transfer the cleared supernatant to a clean 1.5 mL microcentrifuge tube (not supplied). Do not disturb or transfer any of the lysing beads.

Note: In some preparations, a fatty upper layer will form after centrifugation. Transfer of any of the fatty upper layer may reduce RNA yield or clog the column.

7. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge.

Note: A precipitate may form at this point. This will not interfere with the RNA purification. If any sample has lost its volume during homogenization, adjust the volume of ethanol accordingly.

8. Insert an RNA Mini Column into a 2 mL Collection Tube.

9. Transfer 700 µL sample (including any precipitate that may have formed) to the RNA Mini Column.

10. Centrifuge at 10,000 *x g* for 1 minute.

11. Discard the filtrate and reuse the Collection Tube.

12. Repeat Steps 9-11 until all of the sample has been transferred to the column.

13. Add 500 µL Wash Buffer I to the RNA Mini Column.

14. Centrifuge at 10,000 x g for 30 seconds.

15. Discard the filtrate and reuse the Collection Tube.

16. Add 500 µL Wash Buffer II to the RNA Mini Column.

Note: Wash Buffer II must be diluted with 100% ethanol before use. Please refer to "Preparing the reagents" section.

17. Centrifuge at 10,000 x g for 1 minute.

18. Discard the filtrate and reuse the Collection Tube.

19. Repeat Steps 16-18 for a second Wash Buffer II wash step.

20. Centrifuge at maximum speed for 2 minutes to completely dry the RNA Mini Column.

Note: It is important to dry the RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications

21. Transfer the RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

22. Add 40-70 µL Nuclease-free Water.

Note: Make sure to add water directly onto the RNA Mini Column matrix.

23. Centrifuge at top speed for 2 minutes and store eluted RNA at -70°C.

Note: Any combination of the following steps can be used to help increase RNA yield.

- Heat the Nuclease-free Water to 70°C before adding to the column.
- Increase the incubation time to 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
- Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

Cultured Cell Protocol

All centrifugation steps used are performed at room temperature.

1. Determine the proper amount of starting material.

Note: It is critical to use the correct amount of cultured cells in order to obtain optimal yield and purity with the RNA Mini Column. The maximum amount of cells that can be processed with the Total RNA Protocol is dependent on the cell line and its RNA content. The maximum binding capacity of the RNA Mini Column is 100 µg. The maximum number of cells that RNA Lysis Buffer can efficiently lyse is 1×10^7. Use the following table as a guideline to select the correct amount of starting material. If no information regarding your starting material is available, begin with 1×10^6 cells. Based on RNA yield and quality obtained from 1×10^6 cells, the starting amount can be adjusted for the next purification.

Source	Number of cells	RNA Yield (µg):
IC21	1×10^6	12
HeLa	1×10^6	15
293HEK	1×10^6	10
HIN3T3	1×10^6	15

2. Harvest cells using one of the following methods. Do not use more than 1×10^7 cells.

   a. For cells grown in suspension:
      - Determine the number of cells.
      - Centrifuge at 500 *x g* for 5 minutes.

- Aspirate and discard the supernatant.
- Proceed to Step 3.

b. For cells grown in a monolayer:

Note: These cells can either be lysed directly in the cell culture dish or trypsinized and collected as a cell pellet prior to lysis. Cell grown in cell culture flask should always be trypsinized.

- For direct cell lysis:
  - Determine the number of cells
  - Aspirate and discard the cell culture medium.
  - Immediately proceed to Step 3.

  Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate. This will affect the RNA binding conditions of he RNA Mini Column and may reduce RNA Yield.

- To trypsinize and collect cells:
  - Determine the number of cells
  - Aspirate and discard the cell culture medium and wash the cells with PBS.

  Note: Incomplete removal of the cell-culture medium will inhibit trypsin. Multiple washes may be necessary for cells that are difficult to detach.
  - Add 0.1-0.25% Trypsin in a balanced salt solution.
  - Incubate for 3-5 minutes to allow cells to detach. Check cells for detachment before proceeding to the next step.
  - Add an equal volume of cell-culture medium containing serum to inactivate the trypsin.
  - Transfer cells to an RNase-free glass or polypropylene centrifuge tube (not supplied)
  - Centrifuge at 500 *x g* for 5 minutes.
  - Aspirate the supernatant.
  - Immediately proceed to Step 3 below.

  Note: Incomplete removal of the cell-culture medium will inhibit lysis and dilute the lysate. This will affect

the RNA binding conditions of he RNA Mini Column and may reduce RNA Yield.

3. Resuspend cells (do not use more than 1 x 10^7 cells) with RNA Lysis Buffer. Vortex or pipet up and down to mix thoroughly

Note: For pelleted cells, loosen the cell pellet thoroughly by flicking the tube before adding the appropriate amount of Lysis Buffer based on the table below. To directly lyse the cells in the culture dish, add the appropriate amount of Lysis Buffer directly to the dish. Collect the cell lysate with a rubber policemen and transfer the cell lysate into 1.5 mL microcentrifuge tube.

Optional: add 20 µL 2-mercaptoethanol per 1 mL Lysis Buffer before use.

Number of cells	Amount of Lysis Buffer (µL)
< 5 x 10^6	350 µL
5 x 10^6 - 1 x 10^7	700 µL

4. Put the resuspend cells in the recommended Precellys lysing kit tube (see section «Recommended Precellys protocols»).

5. Use the recommended Precellys protocol to disrupt the cells based on the nature of the sample.

6. Centrifuge at ≥10,000 x g for 30 seconds.

7. Transfer the supernatant to a sterile 1.5 mL microcentrifuge tube. Do not disturb or transfer any of the beads.

8. Add 1 volume 70% ethanol. Vortex to mix thoroughly. Do not centrifuge.

Note: A precipitate may form at this point. This will not interfere with RNA purification. If any sample has lost its volume during homogenization, adjust the volume of ethanol accordingly.

9. Insert an RNA Mini Column into a 2 mL Collection tube.

10. Transfer 700 µL sample (including any precipitate that may have formed) to the RNA Mini Column.

11. Centrifuge at 10,000 *x g* for 1 minute.

12. Discard the filtrate and reuse the Collection tube.

13. Repeat Step 10-12 until all the sample has been transferred to the column.

14. Add 500 µL Wash Buffer I to the RNA Mini Column.

15. Centrifuge at 10,000 *x g* for 30 seconds.

16. Discard the filtrate and reuse the Collection tube.

17. Add 500 µL Wash Buffer II to the RNA Mini Column.

Note: Wash Buffer II must be diluted with 100% ethanol before use. Please refer to "Preparing the reagents" section.

18. Centrifuge at 10,000 *x g* for 1 minute.

19. Discard the filtrate and reuse the Collection tube.

20. Repeat Steps 17-19 for a second Wash Buffer II wash step.

21. Centrifuge at maximum speed for 2 minutes to completely dry the RNA mini Column.

Note: It is important to dry the RNA Mini Column matrix before elution. Residual ethanol may interfere with the downstream applications.

22. Transfer the RNA Mini Column to a clean 1.5 mL microcentrifuge tube (not provided).

23. Add 40-70 µL Nuclease-free Water.

Note: Make sure to add water directly onto the RNA Mini Column matrix.

24. Centrifuge at top speed for 2 minutes and store eluted RNA at -70°C.

Note: Any combination of the following steps can be used to help increase RNA yield.

- Heat the Nuclease-free Water to 70°C before adding to the column.
- Increase the incubation time to 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
- Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

Vacuum Protocol

Recommended Settings

The following is required for use with the Vacuum Protocol:

a) Vacuum Manifold
   Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector
b) Vacuum Flask
c) Vacuum Tubing
d) Vacuum Source (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mm Hg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Protocol

Please read through previous sections of this manual before proceeding with this protocol. Steps 1-7 from the Animal Tissue Protocol or steps 1-8 from the Cultured Cell protocol

should be completed before loading the sample to the RNA Mini Column. Instead of continuing with centrifugation, follow the steps below. Do not use more than $1x10^6$ cells or 10 mg tissue for the vacuum protocol.

1. Prepare the vacuum manifold according to manufacturer's instructions
2. Connect the RNA Mini Column to the vacuum manifold.
3. Load the homogenized sample onto the RNA Mini Column.
4. Switch on vacuum source to draw the sample through the column.
5. Turn off the vacuum.
6. Add 500 µL Wash Buffer I to the RNA Mini Column.
7. Switch on vacuum source to draw the Wash Buffer I through the column.
8. Turn off the vacuum.
9. Add 500 µL Wash Buffer II to the RNA Mini Column.

Note: Wash Buffer II must be diluted with 100% ethanol before use. Please refer to "Preparing the reagents" section.

10. Switch on vacuum source to draw the Wash Buffer II through the column.
11. Turn off the vacuum.

12. Repeat Steps 9-11 for a second Wash Buffer II wash step.

13. Remove the column from the vacuum manifold and transfer to a new 2 mL Collection Tube.

14. Centrifuge at maximum speed for 2 minutes to completely dry the RNA Mini Column.

Note: It is important to dry the RNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

15. Transfer the RNA Mini Column into a nuclease-free 1.5 mL microcentrifuge tube.

16. Add 40-70 µL Nuclease-free Water.

Note: Make sure to add water directly to the RNA Mini Column matrix

17. Centrifuge at maximum speed for 2 minutes and store the eluted RNA at -70°C.

Note: Any combination of the following steps can be used to help increase RNA yield.

- Heat the Nuclease-free Water to 70°C before adding to the column.
- Increase the incubation time to 5 minutes.
- Increase the elution volume.
- Repeat the elution step with fresh Nuclease-free Water (this may increase the yield, but decrease the concentration).
- Repeat the elution step using the eluate from the first elution (this may increase yield while maintaining elution volume).

Quantification and Storage of RNA

To determine the concentration and purity of RNA, measure absorbance at 260 nm and 280 nm with a spectrophotometer. One OD unit measured at 260 nm corresponds to 40 µg/mL RNA. Nuclease-free Water is slightly acidic and can dramatically lower absorbance values. We suggest that you dilute the sample in a buffered solution (TE) for spectrophotometric analysis. The A_{260}/A_{280} ratio of pure nucleic acids is 2.0, while an A_{260}/A_{280} ratio of 0.6 denotes pure protein. A ratio of 1.8-2.0 corresponds to 90%-100% pure nucleic acid. Phenol has a maximum absorbance at 270 nm and can interfere with spectrophotometric analysis of DNA or RNA. Store RNA samples at -70°C in water. Under these conditions, RNA is stable for more than a year.

Integrity of RNA

It is highly recommended that RNA quality be determined prior to beginning all downstream applications. The quality of RNA can be best assessed by denaturing agarose gel electrophoresis. The ribosomal RNA bands should appear as sharp, clear bands on the gel. The 28S band should appear to be double that of the 18S RNA band (23S and 16S if using bacteria). If the ribosomal RNA bands in any given lane are not sharp and appear to be smeared towards the smaller sized RNA, it is very likely that the RNA undergone degradation during the isolation, handling, or storage procedure. Although RNA molecules less than 200 bases in length do not efficiently bind to the column matrix, a third

RNA band, the tRNA band, may be visible when a large number of cells are used.

Troubleshooting

Please use this guide to troubleshoot any problems that may arise.

Problem	Cause	Solution
Little or no RNA eluted	RNA remains on the column	Repeat the elution step
	Column is overloaded	Reduce the amount of starting material
Clogged Column	Incomplete	Modify the Precellys protocol used for tissue homogenization
		Reduce the amount of starting material
		Increase the centrifugation time
Degraded RNA	Starting culture problems	Freeze starting material quickly in liquid nitrogen
		Do not store tissue culture cells prior to extraction unless they are lysed first
		Follow protocol closely and work quickly
	RNase contamination	Ensure not to introduce RNase during the procedure
		Check buffers for RNase contamination

Problem in downstream applications	Salt carry-over during elution	Ensure Wash Buffer II has been diluted with 100% ethanol as indicated on bottle
		Wash Buffer II must be stored and used at room temperature
		Repeat wash steps with Wash Buffer II
DNA contamination	DNA contamination	Digest with RNase-free DNase and inactivate DNase by incubation at 65°C for 5 minutes in the presence of EDTA
Low Abs Ratio	RNA diluted in acidic buffer or water	Nuclease-free Water is acidic and can dramatically lower Abs_{260} values. Use TE Buffer to dilute RNA prior to spectrophotometric analysis

Nous contacter

OZYME
Des femmes et des hommes au service de vos recherches

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