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Total RNA Purification Mini Spin Kit

Manual

Kit for isolation of total RNA from animal tissue and cell cultures

Cat#: S5304
Cat#: S5309

Version: 220410

Safety Information

RLys Buffer

Warning
H302, H331, H412
P261, P264, P270, P271, P273, P301+P312+P330, P304+P340, P312

RW1 Buffer

Danger
H225
P210, P233, P280, P303+P361+P353, P403+P235

H225 Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H331** Toxic if inhaled. **H412** Harmful to aquatic life with long lasting effects.

P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. **P233** Keep container tightly closed. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P270** Do not eat, drink or smoke when using this product. **P271** Use only outdoors or in a well-ventilated area. **P273** Avoid release to the environment. **P280** Wear protective gloves / protective clothing/eye protection/face protection. **P301+P312+P330** IF SWALLOWED: Rinse mouth. Call a POISON CENTER / doctor if you feel unwell. **P303+P361+P353** IF ON SKIN (or hair): take off immediately all contaminated clothing. Rinse skin with water/ shower. **P304+P340 P312** IF INHALED: remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER / doctor if you feel unwell. **P403+ P235** Store in a well-ventilated place. Keep cool.

Limitations of Product Use

The use of this kit is strictly limited to research purposes. They are not to be applied for any diagnostic, including human, or drug purposes. This also excludes administration to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the regulatory authorities in the country of use. All due care and attention should be exercised in handling of the materials described in this handbook.

Before using a **Total RNA Purification Kit**, customers and other users should make their own determination that the product is suitable for intended use. They should ensure that they can use the Total RNA Purification Kit product safely and legally. This document does not constitute a warranty or assume any liabilities on behalf of the manufacturer except in writing signed by the manufacturer. Unless otherwise agreed in writing, the exclusive remedy for all claims is replacement of the product or refund of the purchase price at manufacturer's option, and in no event shall the manufacturer be liable for special, consequential, incidental, punitive or exemplary damages.

Average RNA isolation efficiencies from fresh biological material

	Sample Material	Quantity	Yield
	293 HEK cell line	10E6	5µg
	HeLa cell line	10E6	7µg
	Liver	10mg	75µg
	Heart	5mf	10µg
	Brain	5mg	5µg

"Your success is our aim"

For more information: www.genaxxon.com

Related Products

Mini Spin Column DNA Purification Kits	Contents	Cat. No.
Plasmid DNA Mini Purification Kit Purification of plasmid DNA	**50** purifications **250** purifications	S5369.0050 S5369.0250
Endotoxin-free Plasmid DNA Midi Purification Kit Purification of plasmid DNA	**10** purifications **25** purifications	S5365.0010 S5365.0025
Gel Extraction DNA Purification Kit Purification of DNA from gel extraction	**50** purifications **250** purifications	S5374.0050 S5374.0250
PCR and Gel Extraction Kit Purification of DNA from PCR purifications AND purification of DNA from gel extraction	**50** purifications **250** purifications	S5380.0050 S5380.0250
PCR DNA Purification Kit Purification of PCR products	**50** purifications **250** purifications	S5368.0050 S5368.0250
Genomic DNA Blood & Cell Cultures Blood and cell culture	**50** purifications **250** purifications	S5375.0050 S5375.0250
Tissue Genomic DNA Tissue including mouse tail and cells from cell culture	**50** purifications **250** purifications	S5378.0050 S5378.0250
DNA purification columns Separate columns for DNA purification kit	**50** columns **250** columns	S5313.0050 S5313.0250
DNA PLUS purification columns separate columns for purification of Plasmid DNA	**50** columns **250** columns	S5381.0050 S5381.0250
Ceramic Beads for disintegration of tissue	**100** tubes **500** tubes	S5310.0100 S5310.0500
Total RNA Purification Mini Spin Kit for isolation of total RNA from animal and human tissue	**50** purifications **250** purifications	S5304.0050 S5304.0250
Total RNA Purification Mini Spin Kit PLUS Total RNA purification kit with ceramic beads	**50** purifications **250** purifications	S5309.0050 S5309.0250
miRNA Purification Mini Spin Kit for isolation of miRNA from animal and human tissue	**50** purifications **250** purifications	S5305.0050 S5305.0250

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Safety information

- Tissue is treated as a biohazardous material on account of its potential pathogen content or health and life-threatening substances. While working with tissue and cell cultures, compliance with all the safety requirements for working with biohazardous material is essential.
- Conducting the entire isolation procedure in a Class II Biological Safety Cabinet or at a laboratory burner is recommended, as is wearing disposable gloves and a suitable lab coat.
- The use of sterile RNase-free pipette filter tips is recommended.
- Avoid cross-transferral of RNA between columns.
- Guanidine salts residues may from highly reactive compounds when combined with oxidation compounds. In case of spillage, clean area with a detergent/water solution.
- In case of blood spillage, clean area first with detergent/water solution and next with 1% sodium hypochlorite.
- It is strongly recommended to wear a lab coat, disposable gloves and protective goggles when working with chemicals. More detailed information is available in the material safety data sheets, which can be requested from the manufacturer

Storage Conditions and Stability

The Purification Columns of the kit are packed in closed bags and show full performance in this state. They can be stored at room temperature (+15°C - +25°C) for at least 1 year. Opened bags should be closed properly and stored in the refrigerator (+2°C - +8°C).

Protect **RLys Buffer** and **RW1 Buffer** from light.

To avoid evaporation, ensure that buffer bottles are tightly closed before storing.

Guarantee for full performance of the kit as specified in this manual is only valid if storage conditions are followed.

Limited License

The purchase price paid for the **Total RNA Purification Kit** by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended for internal research only by the purchaser. Furthermore, research only use means that the **Total RNA Purification Kit** and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without written approval of the manufacturer.

Separate licenses are available from the manufacturer for the express purpose of non-research use and applications. To inquire about such licenses, or to obtain permission to transfer or use the enclosed material, please contact your local distributor.

Troubleshooting (continued from page 11)

Problem	Possible cause	Comments/suggestions
Purified RNA is degraded	Old material was used	Performing an isolation from fresh tissue is recommended.
	Material was repeatedly frozen/thawed	Avoid subjecting the sample material to repeated freeze/thaw cycles.
	RNases are present.	See “RNase elimination” on page 8 (Recommendations and important notes).
	RNA degraded as a result of over-intensive homogenization	The recommended homogenization conditions should be applied (see page 9).
DNA contamination present	Too much sample material was used	Decrease the amount of sample material. Optionally, the purified RNA sample can be treated with a DNase.
	High amount of DNA present in the material.	The use of on-column DNA digestion is recommended during the RNA purification procedure. While isolating from high amount of material or particular sample type (e. g. brain), incubation with DNase can be prolonged to 15 minutes.
	Inappropriate homogenization	The recommended homogenization conditions should be applied (page 9 and page 10).
	DNase is inactive	Prepare fresh DNase solution. Ensure that DNase solution is stored as recommended.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in the Lysis buffer. Tissue must be first fragmented into as small pieces as possible and homogenized by an appropriate method.
Too low A260:A230 ration of purified RNA yield	**Remaining’s of buffers present in the eluate.**	Ensure that the purification column has been properly dried before elution and no droplets remained on the ring! If necessary, increase centrifugation speed at step 8 of the Isolation Protocol to 18,000 x g. Carefully remove the column from the collection tube.
	Incomplete sample loading.	Make sure that the lysate has passed completely through the RNA Purification Column before proceeding through washing steps. If necessary, increase centrifugation speed at step 4 of the Isolation Protocol.

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Introduction

This **Total RNA Purification kit** is designed for the rapid and efficient purification of high-quality RNA from 1-30mg of tissue (fresh or frozen) and up to 10^7 cultured cells. The isolation protocol and buffer formulations were optimized for high isolation efficiency and purity of RNA. The product is intended for research use only.

Contents of the kits and storage conditions

Content	10 Purifications	50 Purifications	250 Purifications	storage
RLys (Lysis) buffer**	6.6mL	33mL	165mL	RT, store in dark
RW1 buffer (conc.)*** - wash buffer 1	7mL	35mL	175mL	RT, store in dark
RW2 buffer*** - wash buffer 2	3.3mL	17mL	82mL	RT
REB buffer (RNA elution buffer)	1mL	5mL	5 x 5mL	RT
RNA Purification columns	10 pcs	50 pcs	5 x 50 pcs	RT
Collection tubes (2mL)	10 pcs	50 pcs	5 x 50 pcs	RT
Bead-Beating tubes* (S5309 only)	10 pcs	50 pcs	5 x 50 pcs	RT
Manual	1	1	1	

* Refers only to **Total RNA PLUS Purification Kit** (S5309). The bead-beating tubes have a ceramic filling.

** For best efficacy during lysis of difficult material and for protection against RNases it is recommended to add **100% β-mercaptoethanol** (not supplied) to the **RLys Buffer**, to a **final concentration of 1%.** The combined **RLys Buffer** and β-mercaptoethanol will remain stable at 2-8°C for four weeks! Therefore, when isolating in parts, transfer enough of Lysis buffer for one isolation to a separate RNase-free bottle/tube and add β-mercaptoethanol. Marking the bottle after adding β-mercaptoethanol is recommended.

*** Before using for the first time, add appropriate amount of 96%-100% ethanol to the **RW2 Buffer**. For details, see instructions on the bottle label and in the table below. Marking the bottle after adding the alcohol is recommended.

Introduction (continued)

The best efficacy during lysis of difficult material and for protection against RNases it is recommended to add **100% β-mercaptoethanol** to the **RLys Buffer** to **a final concentration of 1%.** The combined **RLys Buffer** and β-mercaptoethanol will remain stable at +2°C to +8°C for four weeks. Therefore, when isolating in parts, transfer enough of the **RLys Buffer** for one isolation to a separate RNase-free bottle/tube and add β-mercaptoethanol. Marking the bottle after adding β-mercaptoethanol is recommended.

Before using for the first time, add appropriate amount of **96-100% ethanol** to the **RW2 Buffer.** For details see the instructions on the bottle label and in the table below. Marking the bottle after adding the alcohol is recommended.

Content	10 Purifications	50 Purifications	250 Purifications	
RLys Buffer**	6.6mL	33mL	165mL	
100% ß-mercaptoethanol	66µL	330µL	1.65mL	
RW2 Buffer	3.3mL	17mL	82mL	
96% - 100% ethanol	13.2mL	68mL	328mL	
Total volume	**16.5mL**	**85mL**	**410mL**	

NOTE: **RW2 Buffer** might be prepared in a smaller volume than given in the table. **RW2 Buffer** should be diluted as follows: 1 volume of **RW2 Buffer** to 4 volumes of ethanol. E. g. for 10 isolations use 2mL **RW2 Buffer concentrate** and 8mL 96-100% ethanol.

NOTE: Protect **RLys and RW1 Buffer** from direct sunlight!

All components can be stored at room temperature (+15°C to +25°C) as long as the appropriate bottles are not opened.

RLys Buffer has to be stored at +2°C to +8°C after β-mercaptoethanol has been added!

To avoid evaporation, ensure that the buffer bottles are tightly closed before storing. Under proper storage conditions, the kit will remain stable for at least 12 months.

Troubleshooting

This guide may help solve problems that may arise. Genaxxon bioscience welcomes comments and suggestions for improvement and supplement of our protocols or any hints on other molecular biology applications. The Genaxxon bioscience team is always pleased to answer any of your questions about our products.

Problem	Possible cause	Comments/suggestions
RNA Purification Column becomes clogged during purification	Inappropriate tissue homogenization	Select the appropriate homogenization conditions (see page 9).
	Tissue and cells remain were transferred into the column.	Pipette the supernatant carefully, without disturbing tissue or cell pellet.
	The purification column is overloaded.	Do not exceed 30mg tissue and 10E7 cells during purification.
Low purified RNA yield	Tissue was incorrectly stored or preserved: RNA degradation.	Store tissue at -80°C° no longer than a year. If a tissue storage buffer was used, ensure its good quality and that the storage conditions are adequate.
	Too little sample material was used.	Take more sample material. A proper amount of the material is dependent on the kind of a cell line/tissue examined and needs to be optimized individually.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in the Lysis buffer. Tissue must be first fragmented into as small pieces as possible and homogenized by an appropriate method.
	Inefficient homogenisation due to an excessive foaming.	Add antifoam Reagent to RLys Buffer according to suppliers instructions.
	The purification column has become clogged.	See “RNA Purification Columns becomes clogged during purification”.
	RNases are present.	See “RNase elimination” page 8. Recommendations and Important Notes.
	RNA is still bound to the column membrane.	Repeat the RNA elution step.
Low purified RNA concentration	Too much of the elution buffer was used.	Decrease **REB** volume to 30-50µL. For a sample concentration it is possible to reload the eluate onto the column and centrifuge again.

Homogenization using a mechanical homogenizer equipped with knives

1. Place the tissue in a 2mL tube, add **100µL RLys Buffer** and carefully homogenize with a sterile homogenizer tip.

2. After homogenization, retrieve tissue remains from the knife tip by washing with **500µL RLys Buffer**. Combine the fractions thus obtained and transfer the entire volume to a new 2mL tube.

3. Continue the isolation from step 2 of the isolation protocol (page 6).

Homogenization using a bead-beating tube

We recommend to use our Total RNA Purification PLUS Kit (S5309), which already contains tubes pre-filled with ceramic beads.

1. **Add 600µL RLys Buffer** to a 2mL ceramic bead-beating tube and suspend the sliced tissue in the buffer.

2. Place the tube in the tissue homogenizer and homogenize for 30 to 60 sec. at 3000 to 4,000 x g.
If necessary, repeat procedure.

NOTE: If a tissue homogenizer is not available, tissue may be homogenized by vortexing using the appropriate 2mL tube adaptor for at least 5 minutes at maximum speed.

3. Continue the isolation from step 2 of the isolation protocol (page 6).

Cell cultures

Quantity: up to 10^7 cells

Sample material: cell suspension or adherent cells, fresh or frozen.

1. Thaw frozen cells at **37°C**. Centrifuge cells suspended in growth medium or PBS buffer, centrifuge in a 15mL centrifugation tube or a 2mL reaction tube at 400 x g. If a compact cell pellet is not formed, wash cells twice with **1mL cold PBS buffer**.

2. Add **600µL RLys Buffer**. Mix by vortexing.
Mix thoroughly by vortexing for 30 sec. and subsequent pipetting.

NOTE: In some cases when cells tend to form syncytia (myoblasts) or tight connections (epithelial cells) or they are high in number (approx. 10^7 cells), it may be difficult to resuspend them in **RLys Buffer**. In such case, pipette carefully, using >1000µL pipette tip or a sterile syringe. Do not use filter tips.

3. Continue the isolation from step 2 of the isolation protocol (page 6).

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Principle

The **Total RNA Purification Kit** utilizes spin columns with membranes which efficiently and selectively bind nucleic acids at high concentration of chaotropic salts.

During the first isolation step, a tissue is homogenized to disintegrate intercellular bonds (epithelial tissue) and fragmentize high molecular protein (muscle or connective tissue). Then the homogenate is lysed with guanidine thiocyanate and detergents. RNases are inactivated by guanidine thiocyanate and β-mercaptoethanol (optional). The homogenate is separated from the undigested tissue/cell that remains after centrifugation. The RNA is bound to the Purification Column membrane by addition of ethanol.

Optional step: **On-column DNase digestion enables removal of remaining genomic DNA.**

The three-step washing stage effectively removes impurities and enzyme inhibitors.

The membrane bound RNA is eluted using a low ionic strength buffer or RNase-free water (pH7.0-9.0) and can be used directly in all downstream applications such as RT-PCR, RT-qPCR, Northern blotting, cDNA synthesis, primer extension, RT-PCR, RNA sequencing, micro arrays, etc.

Additional Materials and Equipment not supplied but required

Needed:

96% - 100% Ethanol
100% ß-mercaptoethanol
1.5 – 2mL RNase free microcentrifuge tubes
Automatic pipettes and pipette tips (RNase-free)
Disposable gloves
Microcentrifuge with rotor for 1.5 – 2mL (>15,000 x g)
Vortex mixer
Freezing racks (<7°C) for 1.5 – 2mL tubes or dish enabling incubation at cooling conditions

May be necessary:

Antifoam agent (S5304.10AF)
Scissors, scalpel
Bead-beating tubes with ceramic filling (Ceramic Beads - cat#: S5310)
Tissue homogenizer for 2mL tubes
Mechanical homogenizer with knives
Thermomixer, shaking orbit of 2mm minimum
50-75mL smooth-stroke mortar with fitted piston
Liquid nitrogen or dry ice
Vortex mixer with a 2mL tube adaptor
Centrifuge with a rotor for 10-15mL tubes (physiological fluids, cell cultures)
3% hydrogen peroxide or <0.5% sodium hypochlorite

Quality Control

The quality of each production batch of the **Total RNA Purification Kit** is tested using standard QC procedures. The purified RNA concentration, quality and stability are evaluated by gel electrophoresis and spectrophotometer. In addition, the functional quality is tested by RT and qPCR.

Product Specifications

Sample material

- Fresh or frozen tissue (stored at -80°C): up to 30mg
- Tissue preserved in RNase inactivating buffer: up to 30mg
- Cell culture: up to 10^7 cells

Efficiency

The typical efficiencies of RNA isolation from fresh biological material are given on page 15.

Binding Capacity

Approximately 230µg RNA

Time required

- 10 – 12 minutes (lysis and homogenisation time not included)
- 15 – 30 minutes for homogenisation in liquid nitrogen.
- 15 – 20 minutes for mechanical homogenisation (Ceramic Beads - cat#: S5310)
- 5 minutes for optional DNase I treatment.

DNA purity

A_{260nm}/ A_{280nm} ratio = 1.9 – 2.1

DNA contamination

All biological material used for RNA isolation also contains DNA. There is no RNA isolation method which guarantees complete DNA removal unless the RNA sample is treated with DNase after isolation. Even slight DNA contamination (several gDNA copies per reaction) may give an additional signal in a quantitative PCR analysis after reverse transcription.

This **Total RNA Purification Kit** provides efficient on-column digestion of DNA during RNA purification as an optional step. DNase I can be removed by **RW1 Buffer**.

We also recommend designing primers which are insensitive to DNA contaminations (primers in adjoining exons or with intron >1.5kbp) for the purposes of qPCR analysis.

Antifoam reagent added to the RIys Buffer

Due to the detergent content of the lysis buffer, foam might be created after homogenization, vortexing or intensive pipetting. To avoid this, **antifoam reagents** can be added to **RLys Buffer**.

RNA clean-up

The Total RNA Purification Kit can be used to clean up RNA after enzymatic reactions, such as labelling, RNA ligation or in-solution DNase digestion. RNA sample is mixed with **RLys Buffer** and ethanol to create conditions that promote selective binding of RNA to the Purification Column. Then, contaminants are effectively washed away ensuring a high-quality RNA recovery.

RNA storage and stability

For long-term storage keep RNA at -80°C or in liquid nitrogen. The high quality and purity of eluted RNA allows to maintain its integrity during a short-term storage from -20°C to room temperature.

Sample Preparation

a. Fresh or frozen solid tissue

Quantity: up to 30mg

Sample material: animal or human tissue.

General procedure, applies to all methods of homogenization

Divide tissue into small fragments with tweezers and scissors or scalpel. Follow one of homogenization methods described below or go to step 1 of the Isolation Protocol (page 6).

Liquid nitrogen, dry ice (LN_2, CO_2)

1. Put tissue frozen in LN_2 or CO_2 in a previously chilled, sterile mortar. Using a chilled piston, carefully, but firmly crush the tissue into small pieces and then, into a pulp.

2. Transfer the powder thus obtained into a 2mL tube containing **600µL Lysis buffer** and go to step 2 of the Isolation Protocol (page 6).

NOTE: After pulping, a thin, sticky layer may be formed, rather than a powder. If this occurs, add 600µL **RLys Buffer** to a mortar and reconstitute the tissue by pipetting and then transfer the lysate into a RNase-free 2mL tube. Remember to retrieve the tissue remains from the piston as well.

RNase elimination

RNases are very active enzymes which do not require any cofactors and are resistant to 15 minutes autoclaving at 121°C. To avoid the degrading effect of the enzymes on RNA, the following recommendations should be followed:

- Always use disposable gloves when working with RNA. Do not get in contact with any items not designed specifically for work with RNA.
- If possible, keep the samples at +2°C to +8°C at all stages of the procedure, including centrifugation. Use decontaminated freezing racks instead of ice to avoid RNase contamination. Keeping RNA after elution in the freezing racks is recommended.
- Disposable plastic ware (tips, tubes, etc.) should be RNase-free or autoclaved at 134°C for 18-20 minutes.
- Reusable plastic ware, glass and porcelain should be soaked overnight in 0.1N NaOH/0.1% DEPC water (or RNase-free water) and then washed with 0.1% DEPC water or RNase-free water). When applicable, glass and porcelain (mortar, pistil), should be parched at 140°C to 150°C for 2 – 4 hours and cooled to room temperature.
- Wipe surfaces, pipettes, centrifuges (wipe the rotor separately) and tube racks with 3% hydrogen peroxide or <0.5% sodium hypochlorite (or any other commercially available RNAse inactivating fluid). Prior to decontamination, test the decontaminant on a small area of the material for possible undesired reactions.

RNA elution

The optimal volume of the **elution buffer REB (RNA Elution Buffer)** used should be chosen in line with the amount of the sample material and the final RNA concentration expected. The use of 30-50µL **REB Buffer** is recommended when **extracting from up to 10mg of tissue or up to 10^6 cells**, increasing the elution buffer volume to 100µL when isolating from 10-30mg of tissue or 10^6 to 10^7 cells.

If a high RNA concentration is desired, the elution volume may be reduced to 50µL. It should be noted that this may reduce the efficiency of the RNA retrieval. It is essential to apply the elution buffer precisely to the centre of the membrane.

When more sample material is to be used for isolation (not recommended as the column can then easily become clogged), full RNA retrieval can be obtained by performing a second elution (100µL). For the second elution, repeat step 10 of the Isolation Protocol (page 7), placing the RNA Purification Column in a new, RNase-free 1.5mL reaction tube.

The elution buffer (**REB Buffer**) does not contain EDTA, which may interfere with some enzymatic reactions.

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Recommendations and important notes

Quantity of starting material

When isolating from more than the recommended quantity of starting material (>30mg, >10^7 cells), divide material into several aliquots/isolations so that each amount (<30mg, <10^7 cells) is isolated with a separate buffer and mini column set.

Exceeded quantities may clog the purification column and/or lower the purity of the isolated RNA.

Sampling and storing the material for RNA isolation

Proper sampling and storing of the biological material prior to RNA isolation is crucial to obtaining a high purity RNA. After sampling, the material should either be preserved by deep freezing at -80°C or in liquid nitrogen or stored at -20°C in RNase inactivating buffer (e.g., RNAlater®, Ambion). Most tissues must be preserved within 30 minutes of sampling.

Tissues rich in RNases (pancreas, liver) **require immediate preservation**!

When isolating from cell cultures, the best results are achieved with fresh material. If storage is unavoidable, discard the supernatant after centrifugation and freeze the cell pellet at -80°C or in liquid nitrogen.

Before getting started

1. Mix well each buffer supplied with the kit. Do not mix the **RLys Buffer** vigorously.
2. Ensure that ethanol has been added to **RW2 Buffer**. If not, add the appropriate amount of **96% - 100% ethanol** (volume values can be found on the bottle labels or in table given on page 2).
3. Prepare 70% ethanol using DEPC-treated water (e.g., M6082).
4. **Examine RLys and RW1 Buffer before use!!!**
   If a sediment occurred in any of them, incubate the **RLys Buffer at 50°C** or **RW1 Buffer at 37°C** mixing occasionally until the sediment has dissolved. Cool at room temperature again.

Optional:

1. Prior to isolation add **100% β-mercaptoethanol** to the **RLys Buffer** to a final **concentration of 1%**. After adding β-mercaptoethanol the Lysis buffer is stable for 4 weeks at +2°C to +8°C. Therefore, when isolating in parts, transfer an appropriate amount of **RLys Buffer** to a separate RNase-free bottle/tube and add β-mercaptoethanol.
2. Prepare freezing rack for storage of the eluted RNA.
3. Prepare DNase I solution according to manufacturer's instructions.
4. Prepare antifoam reagent according to manufacturer's instructions.

RNA Isolation Protocol

1. **Place** the fragmented biological material in a 2mL tube.
   Add 600µL RLys Buffer and vortex for 60 seconds

2. **Centrifuge** for 120 seconds at ≥12000 x g (preferably at 15000 x g).

3. **Transfer** supernatant into an **RNase-free** 1.5 or 2.0mL reaction tube and add **600µL 70% ethanol** to the transferred supernatant.
   Mix by pipetting or vortexing.
   NOTE: For homogenization using bead-beating tubes: carefully pipet the appropriate volume of the supernatant by placing a 200µL pipette tip (N.B.: a 1mL tip may be clogged by the beads) into the filling. Tissue remains should either lie on one side of the tube or at the bottom.

4. **Transfer** up to **700µL of the obtained mixture** into an **RNA Purification Column** placed in a collection tube. Centrifuge for 15 seconds at ≥12000 x g.
   Discard flow-through and re-use the column, together with the collection tube.

5. **Transfer** the **remaining mixture** into the same purification column and centrifuge for 15 seconds at ≥12000 x g.
   Discard flow-through and place the mini column in a new 2mL collection tube.

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6. Optional - On-column DNase treatment

a. Prewash mini column with 500µL **RW2 Buffer** and centrifuge for 60 sec. at ≥12000 x g.
   Discard flow-through and re-use the collection tube.
b. For each isolation mix 90µL **DNase I Reaction Buffer** and 10µL reconstituted **DNase I** (**not included in this kit**). Mix by inverting the tube.
c. Apply 95µL of the above mixture onto the centre of the RNA Purification Column.
   Incubate for 5 minutes at room temperature.
d. Add **600µL RW1 Buffer** and centrifuge for 15 sec. at ≥12000 x g.
   Discard flow-through and re-use the collection tube.
   Proceed with step 8.

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7. **O**mit this step in the case of DNase I treatment / step 6)
Add 700µL RW1 Buffer and centrifuge for 15 sec. at ≥12000 x g.
Discard flow-through and reuse the collection tube.
NOTE: Do proceed with step 8 directly without step 7 if the optional DNase I treatment was done, as described in step 6.

8. **Add 500µL RW2 Buffer** and centrifuge for 15 sec. at ≥12000 x g.
   Repeat this step.

9. Centrifuge for 90 sec. at ≥12000 x g (preferably at 15000 x g). Discard the collection tube and the flow-through and carefully transfer the Purification Column to a RNase-free 1.5mL reaction tube.
   NOTE: RW2 Buffer contains alcohol, which may interfere with some enzymatic reactions and decrease the elution efficiency. It is therefore crucial to remove the alcohol completely from the mini column before elution.

10. **Add 50-100µL elution buffer REB** precisely onto the centre of the purification column membrane. Centrifuge for 60 sec. at ≥12000 x g to elute purified RNA.
    The isolated RNA is ready for use in downstream applications or for storage at -80°C.
    NOTE: Other buffer volumes in the range of 30-50µL may be used. For instructions, see page 8 (RNA elution) (Recommendations and important notes).

RNA Clean-up Protocol (for already isolated RNA)

1. Adjust RNA sample volume to **100µL** by adding RNase-free water to a 1.5 or 2.0mL RNase-free reaction tube.
2. Add **300µL RLys Buffer**.
3. Add **300µL 96-100% ethanol**. Mix well by pipetting or vortexing.
4. Transfer the mixture into a **RNA Purification Column** placed in a collection tube.
   Centrifuge for 15 sec. at ≥12000 x g.
   Discard the flow-through and re-use the collection tube.

   Proceed to **step 7** of the Isolation Protocol (page 7)