

illustra™

# RNAspin Mini RNA Isolation Kit

For the rapid extraction and purification  
of RNA from various samples

Product booklet


Codes:     25-0500-70 (20 purifications)  
              25-0500-71 (50 purifications)  
              25-0500-72 (250 purifications)

See back cover for  
quick reference  
protocol  
card



# Page finder

1. Legal	4
2. Handling	5
2.1. Safety warnings and precautions	5
2.2. Storage	6
2.3. Expiry	6
3. Components	7
3.1. Kit contents	7
3.2. Materials to be supplied by user	8
3.3. Equipment to be supplied by user	9
4. Description	10
4.1. Introduction	10
4.2. The basic principle	11
4.3. Product specifications	13
4.4. Typical output	14
<b>5. Protocols</b>	<b>16</b>
<b>5.1. Preparation of working solutions</b>	<b>16</b>
<b>5.2. Standard protocol for total RNA purification from cultured cells and tissue</b>	<b>17</b>
<b>5.3. Protocol for total RNA purification from non-blood biological fluids</b>	<b>22</b>
<b>5.4. Protocol for total RNA purification from up to <math>10^9</math> bacterial cells</b>	<b>23</b>
<b>5.5. Protocol for total RNA purification from up to <math>5 \times 10^7</math> yeast cells</b>	<b>24</b>
<b>5.6. Protocol for total RNA purification from paraffin embedded tissue</b>	<b>25</b>
<b>5.7. Protocol for total RNA purification from RNAlater® treated samples</b>	<b>26</b>
<b>5.8. Protocol for RNA clean-up from reaction mixtures</b>	<b>26</b>

<b>5.9. Protocol for DNase digestion in solution after purification</b>	<b>27</b>
6. Appendixes	29
6.1. Calculation of RPM from RCF	29
6.2. Preparation and storage of starting materials	29
6.3. Estimation of cell density	31
6.4. Estimation of yield, purity and quality	32
6.5. Troubleshooting guide	33
6.6. Related products	38
7. References	40
 <b>Quick Reference Protocol Card</b>	<b>Back Cover</b>
<b>Tear off sheet containing protocol for the experienced user isolating: Total RNA from cultured cells and tissue (standard protocol)</b>	

# 1. Legal

## Product use restrictions

The components of the **illustra RNAspin Mini RNA Isolation Kit** have been designed, developed and sold for research purposes only. They are suitable for *in vitro* use only. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of **illustra RNAspin Mini RNA Isolation Kit** for a specific application range.

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## 2. Handling

### 2.1. Safety warnings and precautions

**Warning: For research use only.**

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

**Warning: This protocol requires the use of ethanol**

The chaotrope in the Lysis Solution is harmful if ingested, inhaled or absorbed through the skin and can cause nervous system disturbances, severe irritation and burning. High concentrations are extremely destructive to the eyes, skin and mucous membranes of the upper respiratory tract. Gloves should always be worn when handling this solution

Use of this product with cells, tissue, bacteria, yeast and so on should be considered bio-hazardous. Follow appropriate safety procedures while using this kit and when handling RNA isolated from these sources.

Waste effluents from this kit should be decontaminated with bleach or detergent-based method. Decontamination with bleach may be reactive resulting in foam and emission of ammonia gas and should be carried out in an exhaust hood.

## 2.2. Storage

Lyophilized RNase-free DNase I should be stored at +4°C upon arrival.

All other kit components should be stored at room temperature (20–25°C).








## 2.3. Expiry

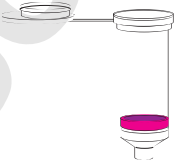
For expiry date please refer to outer packaging label.




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## 3. Components

### 3.1. Kit contents

Identification	Pack Size Cat. No.	20 preps 25-0500-70	50 preps 25-0500-71	250 preps 25-0500-72
 Lysis Solution (Red sticker)		10 ml	25 ml	125 ml
 Wash Buffer I (Yellow sticker)		15 ml	15 ml	80 ml
 Wash Buffer II (Grey sticker)		6 ml Add 24 ml ethanol	12 ml Add 48 ml ethanol	3 × 25 ml Add 100 ml ethanol to each bottle
 Desalting Buffer (Blue sticker)		10 ml	25 ml	125 ml
 DNase Reaction Buffer (Green sticker)		7 ml	7 ml	30 ml
 DNase I, (lyophilized, RNase-free) (Orange sticker)		1 vial Add 230 µl RNase-free H <sub>2</sub> O	1 vial Add 540 µl RNase-free H <sub>2</sub> O	6 vial Add 540 µl RNase-free H <sub>2</sub> O
 RNase-free H <sub>2</sub> O (White sticker)		13 ml	13 ml	60 ml

Identification	Pack Size Cat. No.	20 preps 25-0500-70	50 preps 25-0500-71	250 preps 25-0500-72
 RNAspin Mini Filter		20	50	250

Identification	Pack Size Cat. No.	20 preps 25-0500-70	50 preps 25-0500-71	250 preps 25-0500-72
	RNAspin Mini Column (with collection tube)	20	50	250
	RNAspin Mini Collection Tube	60	150	750
	1.5 ml Microcentrifuge Tube	20	50	250

### 3.2. Materials to be supplied by user

#### Disposables:

1.5 ml RNase-free microcentrifuge tubes

#### Chemicals:

70% and 95–100% ethanol

$\beta$ -Mercaptoethanol ( $\beta$ -ME)

#### Optional chemicals:

Sorbitol/Lyticase buffer (50–100 U Lyticase or Zymolase in 1 M

Sorbitol/100 mM EDTA)

TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8)

Lysozyme

Xylene

3 M Sodium acetate



### 3.3. Equipment to be supplied by user

Microcentrifuge that accommodates 1.5 ml microcentrifuge tubes and can spin at 16 000 × g or higher

Vortex mixer

Rotor-stator homogenizer or Mortar & Pestle

**Optional:**

RNase-free 20-Gauge needle and syringe (1 ml)

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## 4. Description

### 4.1 Introduction

The **illustra™ RNAspin Mini RNA Isolation Kit** is designed for rapid extraction of high quality RNA from various samples.

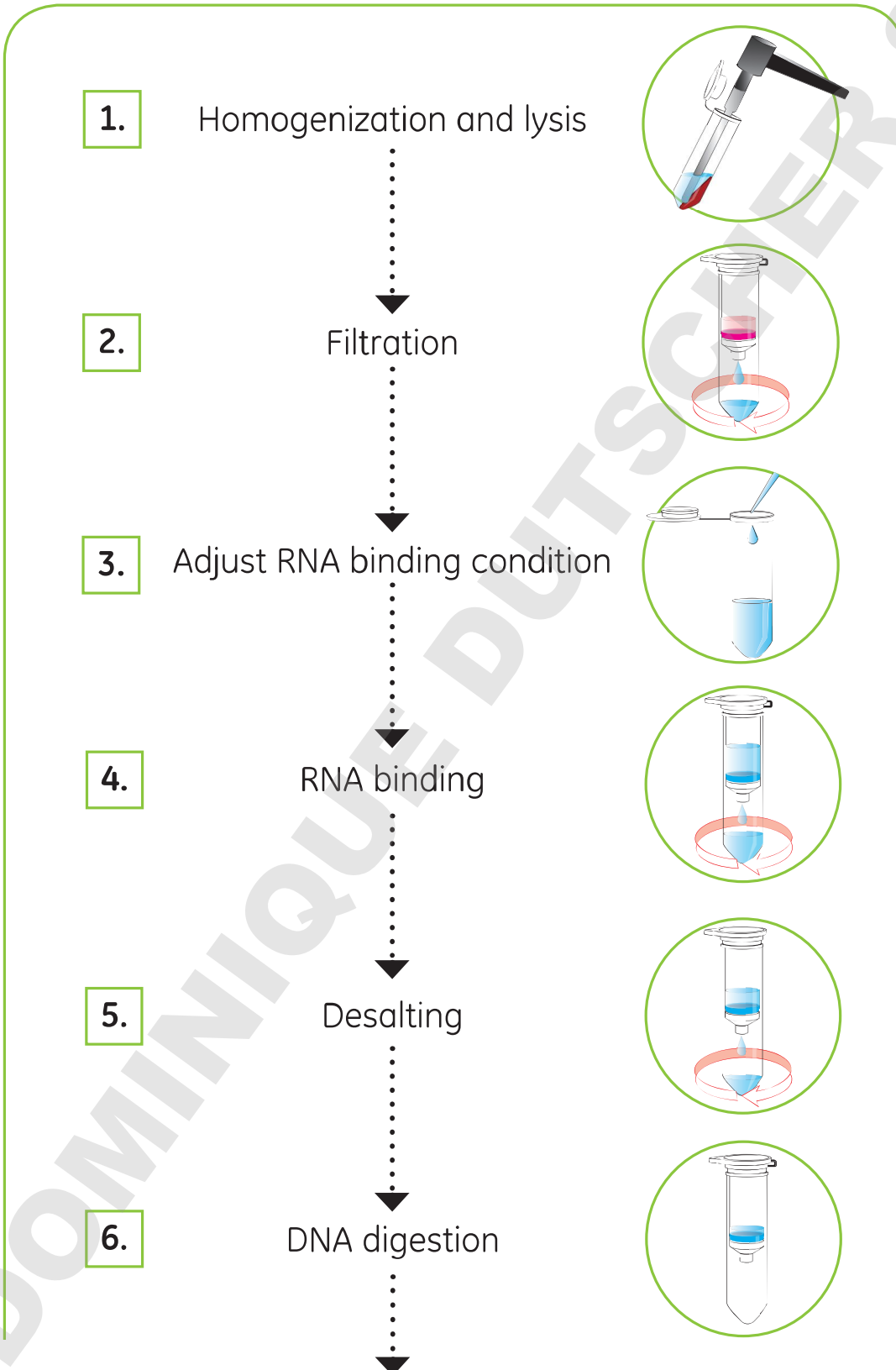
One of the most important aspects in the isolation process is to prevent the degradation of the RNA during the isolation procedure. With the **illustra RNAspin Mini RNA Isolation Kit**, cells are lysed by incubation in a solution containing large amounts of chaotropic ions. This **Lysis Solution** immediately inactivates RNases, which are present in virtually all biological materials. The binding conditions are adjusted to favor adsorption of RNA to the silica membrane. Contaminating DNA, which is also bound to the silica membrane, is removed by the direct application of **DNase I** solution to the silica membrane. Simple washing steps with two different buffers remove salts, metabolites and macromolecular cellular components. Finally, pure RNA is eluted under low ionic strength conditions with RNase-free **H<sub>2</sub>O**.

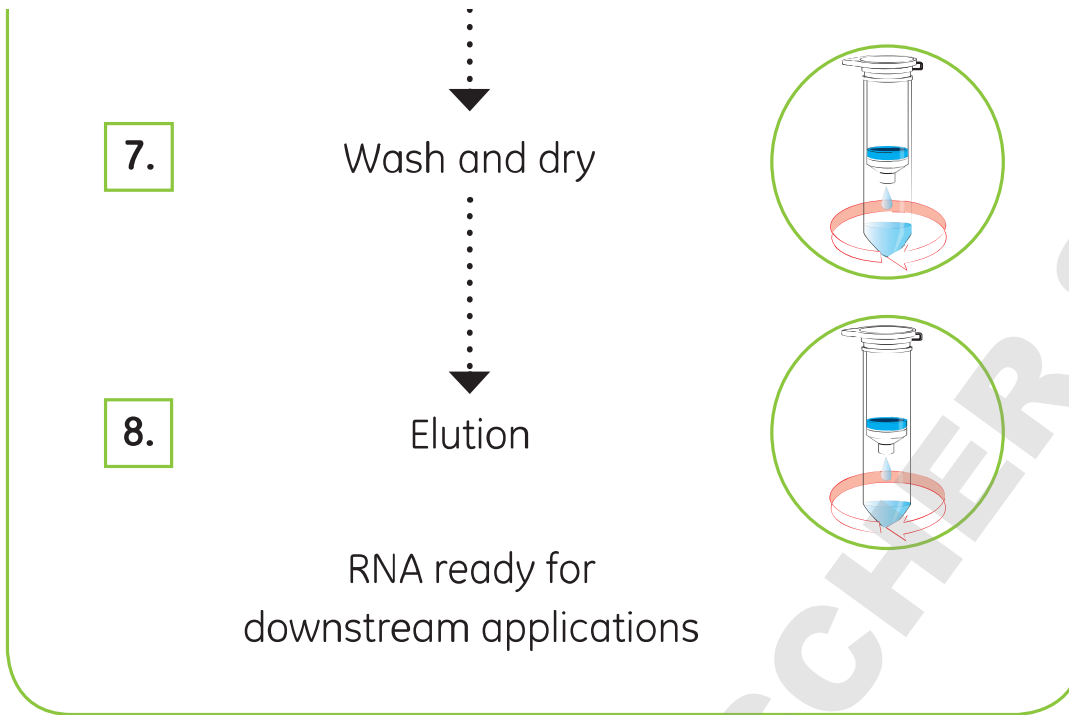
RNA isolation using the **illustra RNAspin Mini RNA Isolation Kit** can be performed at room temperature. However, the eluate should be treated with care because RNA is very sensitive to trace contaminations of RNases, often found on general labware, fingers and dust. To preserve stability, keep the isolated total RNA frozen at -20°C for short-term or -80°C for long-term storage.


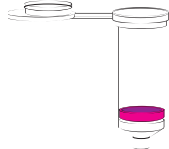
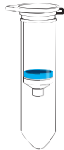
The kit contains sufficient reagents and columns for 20 (25-0500-70), 50 (20-0500-71) or 250 (25-0500-72) purifications.






## 4.2 The basic principle

Use of the **illustra RNAspin Mini RNA Isolation Kit** involves the following steps:





Step	Comments	Component
1. Homogenization and Lysis	Procedure depends on sample type	Lysis Solution 
2. Filtration	Reduce viscosity and clear the lysate	RNAspin Mini Filter 
3. Adjust RNA binding condition	Create appropriate binding conditions that favor adsorption of RNA to the silica membrane	Ethanol
4. RNA Binding	Add 70% ethanol, then bind to RNAspin Mini Column	RNAspin Mini Column / Collection Tube 

Step	Comments	Component
5. Desalting	Reduce the salt concentration and prepare for DNA digestion	Desalting Buffer 
6. DNA digestion	Digest the DNA on the column	DNase I 
7. Wash and dry	A combined washing /drying step to remove contaminants from the membrane-bound RNA. These steps use ethanol-based buffers	Wash Buffer I & II  
8. Elution	Elute high quality RNA in RNase-free water	RNase-free H <sub>2</sub> O 

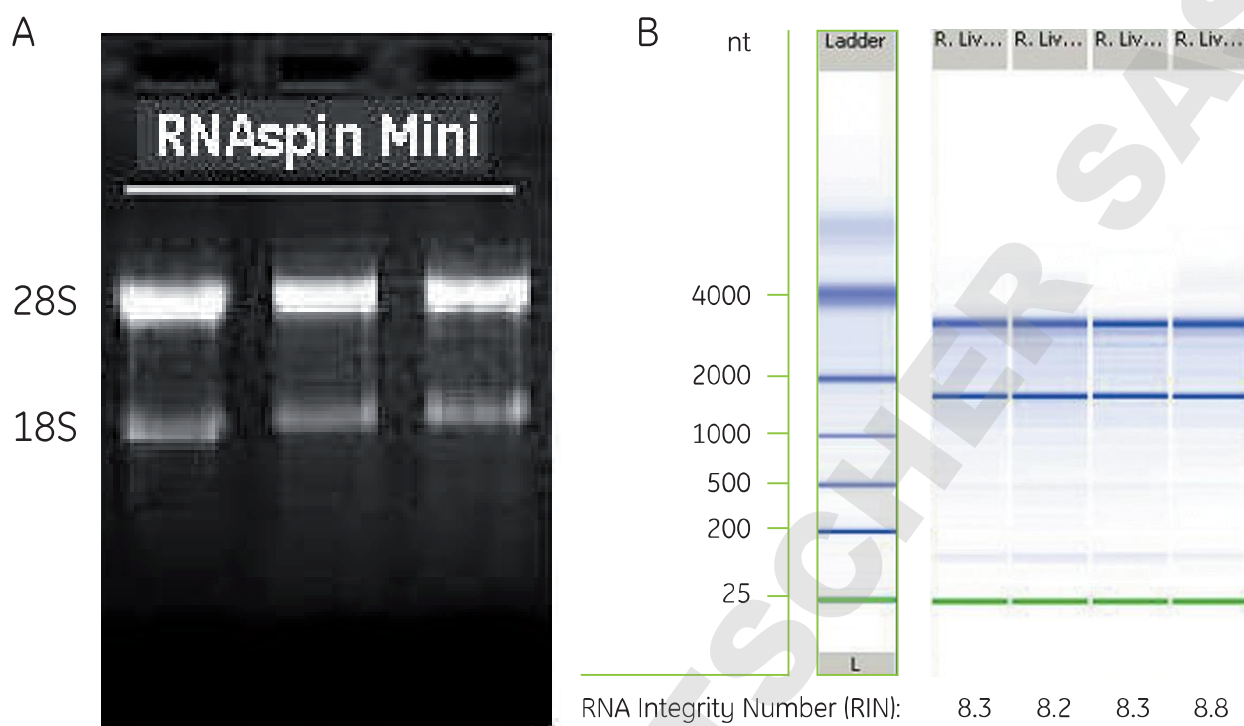
### 4.3. Product specifications

	Tissue	Cell culture
Sample size	up to 30 mg tissue	up to 5 × 10 <sup>6</sup> cells
Typical yield*	up to 70 µg	up to 70 µg
Elution volume	40–120 µl	
Effective binding capacity	100 µg	
RNA integrity	sharp rRNA bands with no substantial degradative bands visible 28S:18S = ~2:1 RNA Integrity Number (RIN) values ≥ 7	
RNA purity	$A_{260}/A_{280} = 1.8-2.2$	
Time/Prep**	< 30 min/6 preps	

\*Actual yields will vary depending on sample and the growth phase.

\*\*Actual time/prep will vary depending on user's experience with the protocol.

## 4.4. Typical output



**Fig 4.1.** The **illustra RNAspin Mini RNA Isolation Kit** produces high quality RNA

rRNA bands are sharp, with the 28S band being about twice as intense as the 18S band, and with good RNA Integrity Number (RIN) values. (A) Total RNA from  $10^6$  HeLa cells was isolated with **illustra RNAspin Mini RNA Isolation Kit** and separated by gel electrophoresis on a 1.2% formaldehyde agarose gel; (B) Total RNA from rat liver was isolated with **illustra RNAspin Mini RNA Isolation Kit** and evaluated using the Agilent 2100 Bioanalyzer.

The **illustra RNAspin Mini RNA Isolation Kit** allows for the isolation of pure RNA with an  $A_{260}/A_{280}$  ratio generally exceeding 1.9 (measured in 5 mM Tris-HCl buffer, pH 8.5). Even biological samples that are sometimes difficult to process will yield high quality RNA. These include mouse tissue (liver, brain), different tumor cell lines, *Streptococci*, and *Actinobacillus pleuropneumoniae*. Note that this kit is not suitable for isolation of RNA from blood.

The isolated RNA is ready to use for downstream applications like Quantitative Reverse Transcriptase-PCR (RT-qPCR), Primer Extension, RNase Protection Assays, cDNA Synthesis and Microarray Analysis.

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# 5. Protocols

## Use of icons



This icon is used to highlight particularly critical steps within the protocol that must be adhered to. If this advice is not followed, it will have a detrimental impact on results.



This icon is used to highlight technical tips that will enhance the description of the step. These tips may indicate areas of flexibility in the protocol or give a recommendation to obtain optimum performance of the kit.

## 5.1. Preparation of working solutions

See “Materials to be supplied by user” at Section 3.2.

### DNase I



Avoid vigorous mixing of the **DNase I** enzyme because it is sensitive to mechanical agitation.

Add the indicated volume of RNase-free **H<sub>2</sub>O** to the **DNase I** vial and incubate for 1 min at room temperature. Gently swirl the vial to completely dissolve the **DNase I**. Dispense into aliquots and store at -20°C. The frozen working solution is stable for 6 mo. Do not freeze/thaw the aliquots more than three times.

### Wash Buffer II

Add the indicated volume of 96–100% ethanol to the **Wash Buffer II** concentrate. Store **Wash Buffer II** at room temperature (20–25°C) for up to 1 yr.



## 5.2. Standard protocol for total RNA purification from cultured cells and tissue

### 1. Homogenization and lysis

#### 1.1. Tissue homogenization and lysis using a mortar and pestle.


- a. Dispense sufficient liquid nitrogen to cover the bottom of a mortar. Immediately place tissue (up to 30 mg) in liquid nitrogen, and grind the sample to a fine powder in the presence of liquid nitrogen.




Disrupt tissue with mortar and pestle

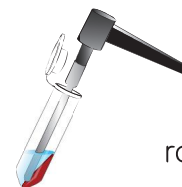


Make sure that the sample does not thaw during or after weighing and grinding. See Section 6.2 for "Preparation and storage of starting materials".

- b. Add 350  $\mu\text{l}$  **Lysis Solution**  and 3.5  $\mu\text{l}$   $\beta$ -mercaptoethanol. Grind further with the pestle until no tissue pieces visible.

#### 1.2. Tissue homogenization and lysis using a rotor-stator.

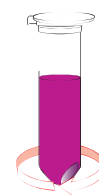
- a. Add 350  $\mu\text{l}$  **Lysis Solution**  and 3.5  $\mu\text{l}$   $\beta$ -mercaptoethanol to frozen tissue (up to 30 mg).
- b. Disrupt the tissue with rotor-stator. Visually inspect and no tissue piece should be visible.



Disrupt tissue with rotor-stator


#### 1.3. Homogenization and lysis of cultured cells.

- a. Pellet up to  $5 \times 10^6$  cultured cells. Centrifuge at  $5000 \times g$  for 1 min. Completely remove the supernatant by aspiration.



Up to  $5 \times 10^6$  cells  
 $5000 \times g$ ,  
1 min

b. Wash the pellet with 500  $\mu$ l PBS. Centrifuge at 5000  $\times$  g for 1 min. Completely remove the supernatant by aspiration.

c. Add 350  $\mu$ l **Lysis Solution**  and 3.5  $\mu$ l  $\beta$ -mercaptoethanol. Pipet up-and-down to re-suspend the cell pellet and lyse the cell directly.



350  $\mu$ l  
Lysis  
Solution  
3.5  $\mu$ l  
 $\beta$ -ME

## 2. Filtration

Reduce viscosity and clear the lysate by filtration through **RNAspin Mini Filter**



RNAspin  
Mini Filter

a. Place **RNAspin Mini Filter** in a collection tube.



b. Apply the lysate to the **RNAspin Mini Filter**.



load lysate

c. Centrifuge at 11 000  $\times$  g for 1 min.



11 000  $\times$  g,  
1 min

d. Discard the **RNAspin Mini Filter**. Transfer filtrate, avoiding aspirating any formed pellet, to a new 1.5 ml RNase-free microcentrifuge tube (not included).

Alternatively, the lysate may be passed  $\geq 5$  times through a 0.9 mm needle (20 gauge) fitted to a syringe.



To process higher amount of cells ( $> 1 \times 10^6$ ) or tissue ( $> 10$  mg), the lysate should first be sheared using the 0.9 mm needle (20 gauge) and then proceed to filtration through the **RNAspin Mini Filter**.

### 3. Adjust RNA binding condition

Add 350  $\mu$ l ethanol (70%) and mix by vortexing, twice for 5 s each.



350  $\mu$ l  
70% ethanol



After the addition of ethanol, a stringy precipitate may become visible. This will not affect RNA isolation. Be sure to load all of the precipitate onto the column.

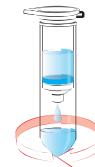
### 4. RNA binding

a. Pipet lysate up-and-down 2 to 3 times, then load the lysate onto the **RNAspin Mini Column**.



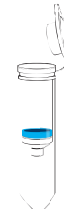
Load lysate

b. Centrifuge for 30 s at 8000  $\times$  g.



8000  $\times$  g,  
30 s

c. Transfer the column to a new collection tube.



Column  
in new  
collection  
tube



Maximal loading capacity of **RNAspin Mini Column** is 750  $\mu$ l. Repeat the procedure if larger volumes are to be processed.

### 5. Desalting

a. Add 350  $\mu$ l **Desalting Buffer** .



350  $\mu$ l  
Desalting  
Buffer



b. Centrifuge at 11 000  $\times$  g for 1 min to dry the membrane. Discard the flowthrough and return the **RNAspin Mini Column** to the collection tube.



11 000  $\times$  g,  
1 min

Salt removal will make the subsequent DNase digestion much more effective. If the column outlet has come into contact with the flowthrough for any reason, discard the flowthrough and centrifuge again for 30 s at 11 000 × g.

## 6. DNA digestion

a. Prepare DNase I reaction mixture in a sterile microcentrifuge tube for each isolation, add 10 µl reconstituted **DNase I**  to 90 µl **DNase Reaction Buffer** . Mix by flicking the tube.

b. Apply 95 µl of DNase I reaction mixture directly onto the center of the silica membrane of the column.



95 µl  
of  
DNase I  
reaction  
mixture

c. Incubate at room temperature for 15 min.



Room  
temperature  
15 min

## 7. Wash and dry

a. 1st wash

Add 200 µl **Wash Buffer I**  to the **RNAspin Mini Column**.



200 µl  
Wash  
Buffer I

Centrifuge for 1 min at 11 000 × g. Place the column into a new collection tube.



11 000 × g,  
1 min

**Wash Buffer I**  will inactivate DNase.

b. 2nd wash

Add 600  $\mu$ l **Wash Buffer II**  to the **RNAspin Mini Column**.

Centrifuge for 1 min at 11 000  $\times$  g.

Discard flowthrough and place the column back into the collection tube.

c. 3rd wash

Add 250  $\mu$ l **Wash Buffer II**  to the **RNAspin Mini Column**.

Centrifuge for 2 min at 11 000  $\times$  g to dry the membrane completely.

Place the column into a nuclease-free **1.5 ml Microcentrifuge Tube** (supplied).



If for any reason the liquid level in the collection tube has reached the **RNAspin Mini Column** after centrifugation, discard flowthrough and centrifuge again.



Check that no residual flowthrough remains in the column outlet. If any remains, re-centrifuge.



Column to new collection tube



600  $\mu$ l Wash Buffer II



11 000  $\times$  g, 1 min



250  $\mu$ l Wash Buffer II




11 000  $\times$  g, 1 min





Column to 1.5 ml Microcentrifuge Tube

## 8. Elution

Elute the RNA in 100  $\mu\text{l}$  RNase-free  $\text{H}_2\text{O}$   and centrifuge at 11 000  $\times$  g for 1 min.



It is possible to adapt the elution protocol for the subsequent applications of interest.

- If high yield is required, do second elution with another 100  $\mu\text{l}$  RNase-free  $\text{H}_2\text{O}$  . About 90–100% of bound RNA will be eluted.
- If higher concentration is required, elute with 40  $\mu\text{l}$  RNase-free  $\text{H}_2\text{O}$  . However, the overall yield will decrease when using small elution volume.
- If both high yield and high concentration are required, elute with the standard elution volume and apply the eluate onto the column for re-elution.



Eluted RNA should be immediately placed on ice to prevent potential degradation. Keep at  $-20^\circ\text{C}$  or  $-80^\circ\text{C}$  for short-term or long-term storage, respectively.

## 5.3. Protocol for total RNA purification from non-blood biological fluids

For serum, culture medium and so on.

### 1. Lysis

Add 350  $\mu\text{l}$  **Lysis Solution**  to 100  $\mu\text{l}$  of sample and vortex vigorously. No need for homogenization or filtration of the lysate



**Proceed directly with Step 3 of Section 5.2.**



100  $\mu\text{l}$   
RNase-free  
 $\text{H}_2\text{O}$

11 000  $\times$  g,  
1 min

## 5.4. Protocol for total RNA purification from up to $10^9$ bacterial cells




Because of the higher relative concentration of genome equivalents in a nucleic acid preparation of bacteria compared with eukaryotic material, it may be necessary to use a lower amount of bacteria cells for the preparation.

### 1. Homogenization and lysis

- a. For purification of RNA from Gram-negative bacteria, resuspend the cell pellet in 100  $\mu$ l TE buffer containing 0.2 mg/ml Lysozyme by vigorously vortexing. Incubate at 37°C for 10 min.

For preparation of RNA from Gram-positive bacteria, resuspend the cell pellet in 100  $\mu$ l TE buffer containing 2 mg/ml Lysozyme. It may be necessary to optimize incubation time and Lysozyme concentration, according to the bacterial strain.

See “Material to be supplied by user” at Section 3.2.

- b. Add 350  $\mu$ l **Lysis Solution**  and 3.5  $\mu$ l  $\beta$ -mercaptoethanol. Mix by vortexing.


**Proceed with Step 2 of Section 5.2.**

## 5.5. Protocol for total RNA purification from up to $5 \times 10^7$ yeast cells



Due to the higher relative concentration of genome equivalents in a nucleic acid preparation of yeasts compared with cultured cells or tissue material, it may be necessary to use a lower amount of yeast cells for the preparation.

### 1. Homogenization and lysis

- a. Harvest 2–5 ml of culture. Centrifuge at  $5000 \times g$  for 10 min. Discard culture media.
- b. Resuspend pellet in Sorbitol/Lyticase buffer (See “Materials to be supplied by user” at Section 3.2) and incubate at  $30^\circ\text{C}$  for 30 min. Pellet the resulting spheroplasts by centrifugation at  $1000 \times g$  for 10 min. Remove the supernatant.
- c. Add 350  $\mu\text{l}$  **Lysis Solution**  and 3.5  $\mu\text{l}$   $\beta$ -mercaptoethanol to the suspension and vortex vigorously.



It may be necessary to optimize incubation time and Lyticase/ Zymolase concentration, according to the yeast strain.

**Proceed with Step 2 of Section 5.2.**



## 5.6. Protocol for total RNA purification from paraffin embedded tissue

### 1. Xylene wash

Put 10 mg of finely minced tissue into a 1.5 ml RNase-free microcentrifuge tube. Add 300  $\mu$ l xylene and incubate 5 min with constant mixing at room temperature. Centrifuge at full speed (16 000  $\times$  g) for 3 min to pellet the tissue. Remove the supernatant.

Repeat twice for a total of three xylene washes.

### 2. Ethanol wash

Add 300  $\mu$ l 96% ethanol to the tube and incubate for 5 min at room temperature with constant mixing. Centrifuge at full speed (16 000  $\times$  g) for 3 min to pellet the tissue. Discard the supernatant.

Repeat for a total of two ethanol washes


**Proceed with Step 2 of Section 5.2.**

## 5.7. Protocol for total RNA purification from *RNAlater*<sup>®</sup> treated samples

### 1. Sample Preparation

Remove *RNAlater* solution. Cut an appropriate amount of tissue.

### 2. Homogenization and lysis

Add 350  $\mu$ l **Lysis Solution**  and 3.5  $\mu$ l  $\beta$ -mercaptoethanol to the sample, disrupt the tissue.

Proceed with Step 2 of Section 5.2.

## 5.8. Protocol for RNA clean-up from reaction mixtures

### 1. Clean-up

Add 3.5 volumes of **Lysis Solution**  per 1 volume of sample, and vortex to mix.

Maximal loading capacity of **RNAspin Mini Column** is 750  $\mu$ l. Repeat the procedure if larger volumes are to be processed.



In order to load all of the sample in one step with subsequent steps, do not use more than 90  $\mu$ l of sample for the **illustra RNAspin Mini RNA Isolation Kit**.

### 2. Adjust RNA binding condition

Add 3.5 volumes of ethanol (95–100%) per 1 volume of sample (as Step 1) to the lysate, mix by vortexing.



Proceed with Step 4 of Section 5.2.

## 5.9. Protocol for DNase digestion in solution after purification



For downstream applications, which require completely undetectable DNA level or lowest residual content of DNA, DNase digestion in solution after purification is recommended. Stringent RNase control and subsequent re-purification of the RNA are usually required in order to remove buffer, salt, DNase and digested DNA.

### 1. DNase digestion

- Prepare DNase working solution by premixing reconstituted **DNase I**  with **DNase Reaction Buffer**  at ratio of 1 to 10.
- For 1 volume of purified RNA, add 1/10 volume of DNase working solution.
- Incubate for 10 min at 37°C.

### 2. Re-purification of RNA by ethanol precipitation

- Add 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 96–100% ethanol to 1 volume of DNase treated sample. Mix thoroughly.
- Incubate at -20°C or 4°C for 10 min to 3 h depending on the RNA concentration. Usually, the lower RNA concentration needs the longer incubation time.
- Centrifuge at full speed (16 000 × g) for 10 min. Remove the supernatant.

- d. Wash the pellet with 70% ethanol. Dry RNA pellet and resuspend RNA in RNase-free  $\text{H}_2\text{O}$



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## 6. Appendices

### 6.1. Calculation of RPM from RCF

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

$$\text{RPM} = 1000 \times \sqrt{\text{RCF}/1.12 r}$$

Where RCF = relative centrifugal force; r = radius in mm measured from the center of the spindle to the bottom of the rotor bucket; and RPM = revolutions per min.

For example, if an RCF of 735 × g is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3000.

### 6.2. Preparation and storage of starting materials

RNA is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Therefore it is important that samples are flash frozen in liquid N<sub>2</sub> immediately, and stored at -70°C, stored in a stabilizing agent, or processed as soon as possible. Samples can be stored in **Lysis Solution** after disruption at -70°C for up to 1 yr, at +4°C for up to 24 h or up to several hours at room temperature. Frozen samples are stable up to 6 mo. Frozen samples in **Lysis Solution** should be thawed slowly before starting with the isolation of total RNA.

**Cultured cells** are collected by centrifugation and directly lysed by adding **Lysis Solution** according to Step 1 of Section 5.2 as the standard protocol.

**Animal tissues** are often solid and must therefore be broken up mechanically as well as lysed. Depending on the disruption method, the viscosity of the lysed sample has to be reduced further for

optimal results. It is essential for efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption and that the viscosity of the sample is reduced by homogenization.

#### **a. Mortar and pestle**

The most commonly used technique for disruption of animal tissue is grinding with a mortar and pestle. Grind the sample to a fine powder in the presence of liquid N<sub>2</sub>. Take care that the sample does not thaw during or after weighing or grinding and add an appropriate aliquot of **Lysis Solution** containing β-mercaptoethanol and mix immediately.

This technique tends to be a gentle cell disruption method (significantly gentler than sonication). However, this technique is laborious, slow and usually inefficient. It also generates heat so cooling is necessary.

#### **b. Rotor-stator homogenizer**

Thawing of undisrupted animal tissue should be exclusively done in the presence of **Lysis Solution** and β-mercaptoethanol during simultaneous mechanical disruption, for example, with a rotor-stator homogenizer. This ensures that the RNA is not degraded by RNases before the preparation has started.

The spinning rotor simultaneously disrupts and homogenizes the sample by mechanical shearing within seconds up to minutes (homogenization time depends on sample). Take care to keep the rotor tip submerged in order to avoid excess foaming. The RNA yield with this method could be much higher than the method with mortar and pestle.

**Bacteria and yeasts** have to be incubated in Lysozyme or Lyticase/Zymolase solutions, respectively (see protocols in Section 5.4 and 5.5). By this treatment, the robust cell walls of these organisms are digested or at least weakened, which is essential for effective cell lysis by **Lysis Solution**. For microorganisms with extremely resistant

cell walls - like some Gram-positive bacterial strains - it may be necessary to optimize the conditions of the treatment with Lytic enzymes or the cultivation conditions.

### 6.3. Estimation of cell density

Do not exceed  $5 \times 10^6$  cells per sample when purifying total RNA from the cultured mammalian cells. Total RNA yield drops when silica columns begin to experience clogging (seen at  $1 \times 10^7$  cells). Cell density should be estimated using an automated cell counter (e.g. Coulter) or counted under a microscope using a standard hemocytometer (e.g. Hausser Scientific, catalog number 1483). If sample total cell count exceeds  $5 \times 10^6$ , split sample into two and proceed with two total RNA preparations. Below are guidelines for measuring cell density using a hemocytometer.

1. Clean a hemocytometer and the short coverslip thoroughly and wipe clean with ethanol.
2. If working with adherent cells, trypsinize the cells and wash once with PBS.
3. Resuspend cells in appropriate volume of PBS or culture medium to yield roughly  $1 \times 10^6$  cell/ml. Make sure cells are completely resuspended without any visible clumps.
4. Add 10  $\mu$ l of resuspended cells to two chambers of hemocytometer (under a small coverslip) making sure the solution spreads completely under the coverslip (by capillary action).
5. Place the hemocytometer under a light microscope, focus on the cells using lowest magnification and begin counting cells only at the four corner  $1 \text{ mm}^2$  squares and the middle  $1 \text{ mm}^2$  square. Aim to have 50–100 cells per square-grid. If cell count is  $> 150/\text{grid}$ , it is advisable to dilute the cells, clean the hemocytometer and re-count cells.

6. Add the number of cells together from the five 1 mm<sup>2</sup> squares, and multiply by 1000 to give the number of cells/ml in PBS or culture medium.

#### 6.4. Estimation of yield, purity and quality

The concentration of purified RNA should be determined by UV spectrophotometer ( $A_{260}$ ). The reliable range of  $A_{260}$  data should be determined for individual spectrophotometers. Generally, for spectrophotometers with a 1 cm path length,  $A_{260}$  readings should lie between 0.1 and 1.0 and appropriate dilutions (4 to 40 ng/ $\mu$ l) should be analyzed.

1 OD unit ( $A_{260}$ ) is equivalent to approximately 40  $\mu$ g/ml RNA.

Yield =  $A_{260} \times 40 \mu\text{g/ml} \times 0.1 \text{ ml}$  = the total  $\mu$ g of purified RNA in the sample.

The UV spectrophotometric ratio  $A_{260}:A_{280}$  provides information regarding the purity of RNA. A purity ratio from 1.8 to 2.2 indicates that the RNA is pure.

The quality of purified RNA could be accessed by Agilent Bioanalyzer (1). The RNA concentration needs to be in the range of 100–500 ng/ $\mu$ l for reliable RIN and 28S:18S ratio. See “*Product specifications*” at Section 4.3.



## 6.5. Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact GE Healthcare technical services.

Alternatively visit <http://www.gehealthcare.com/illustra>

### **Problem: RNA is degraded/no RNA obtained**

<b>Possible cause</b>	<b>Suggestions</b>
<i>RNase contamination</i>	<ul style="list-style-type: none"><li>• Create an RNase-free working environment. Wear gloves during all steps of the procedure, and change gloves frequently. Use of sterile, disposable polypropylene tubes is recommended. Keep tubes closed whenever possible during the preparation. Glassware should be oven-baked for at least 2 hours at 250°C before use.</li></ul>

### **Problem: Poor RNA quality or yield**

<b>Possible cause</b>	<b>Suggestions</b>
<i>Reagents not prepared, stored or applied properly</i>	<ul style="list-style-type: none"><li>• Reagents not properly reconstituted. Add the indicated volume of RNase-free <b>H<sub>2</sub>O</b> to <b>DNase I</b> vial or 96–100% ethanol to the concentrate of <b>Wash Buffer II</b>, then mix.</li><li>• Sample and reagents have not been mixed completely. Always vortex vigorously after each reagent has been added.</li><li>• Ethanol was not added after lysis.</li></ul>

## **Problem: Poor RNA quality or yield, continued**

### **Possible cause**

### **Suggestions**

*Reagents not prepared, stored or applied properly, continued*

Binding of RNA to the silica membrane is only effective in the presence of ethanol.

- Reconstitute and store lyophilized **DNase I** according to instructions given in Section 5.1.
- Store other kit components at room temperature. Storage at low temperatures may cause salt precipitation.
- Keep bottles tightly closed in order to prevent evaporation or contamination.

*Suboptimal elution*

- Be sure that all of the water gets into contact with the silica membrane. No water drops should stick to the walls of the columns. The membrane has to be wetted completely.
- Ionic strength and pH influence  $A_{260}$  absorption as well as ratio  $A_{260}/A_{280}$ ; thus, for absorption measurement, use 5 mM Tris-HCl pH 8.5 as diluent.

*Sample material*

- Sample material not stored properly. Whenever possible, use fresh material. If this is not possible, flash freeze the samples in liquid  $N_2$  or treat with a stabilizing agent. Samples should

## **Problem: Poor RNA quality or yield, continued**

<b>Possible cause</b>	<b>Suggestions</b>
<i>Sample material, continued</i>	<p>always be kept at -80°C. Never allow tissues to thaw before addition of <b>Lysis Solution</b>. Perform disruption of samples in liquid N<sub>2</sub>, if possible.</p> <ul style="list-style-type: none"><li>• Insufficient disruption and/or homogenization of starting material. Ensure thorough sample disruption and use <b>RNAspin Mini Filter</b> for easy clean-up of disrupted starting material.</li><li>• Too much starting material may lead to <b>RNAspin Mini Column</b> clogging and reduced RNA quality or yield. For clogging issues, see below. RNA quality and yield problems relating to too much sample material may be addressed by decreasing the amount of starting material and/or increasing the volumes of <b>Wash Buffer I</b> and <b>Wash Buffer II</b>.</li></ul>

## **Problem: Clogged RNAspin Mini Column**

<b>Possible cause</b>	<b>Suggestions</b>
<i>Sample material</i>	<ul style="list-style-type: none"><li>• Use the <b>RNAspin Mini Filter</b> to reduce the risk of clogging on the <b>RNAspin Mini Column</b>.</li><li>• To prevent clogging due to too much starting material, reduce the sample amount, increase the time for the centrifugation steps, and/or increase</li></ul>

## **Problem: Clogged RNAspin Binding Column, continued**

<b>Possible cause</b>	<b>Suggestions</b>
<i>Sample material, continued</i>	the volume of <b>Lysis Solution</b> . If clogging still occurs during the run, take the remaining lysate off the <b>RNAspin Mini Column</b> , discard it, and proceed with the desalting step.

## **Problem: Contamination of RNA with genomic DNA**

<b>Possible cause</b>	<b>Suggestions</b>
<i>DNase I not active</i>	<ul style="list-style-type: none"><li>• Reconstitute and store lyophilized <b>DNase I</b> according to instructions given in Section 5.1.</li></ul>
<i>DNase solution not properly applied</i>	<ul style="list-style-type: none"><li>• Pipet <b>DNase I</b> solution directly onto the center of the silica membrane.</li></ul>
<i>Too much cell material used</i>	<ul style="list-style-type: none"><li>• Reduce quantity of cells or tissue used</li></ul>
<i>DNA detection system too sensitive</i>	<ul style="list-style-type: none"><li>• The amount of DNA contamination is significantly reduced during the on-column <b>DNase I</b> digestion. Residual DNA may still be present; therefore in very sensitive applications, it might be possible to detect DNA.  As part of the product QC process, the <b>illustra RNAspin Mini RNA Isolation Kit</b> is assessed for DNA contamination by the following procedure:</li></ul>

## **Problem: Contamination of RNA with genomic DNA, continued**

<b>Possible cause</b>	<b>Suggestions</b>
<i>DNA detection system too sensitive, continued</i>	<p>1 × 10<sup>6</sup> HeLa cells are subjected to RNA isolation according to the protocol. RNA eluate is used as template for PCR detection of a 1 kb fragment in a 30-cycle reaction.</p> <p>Generally, no PCR fragment is obtained if <b>DNase I</b> is applied. However, a strong PCR fragment is obtained if the DNase is omitted.</p> <p>The potential for DNA contamination detection by PCR increases with:</p> <ul style="list-style-type: none"><li>- increasing the number of DNA copies per preparation</li><li>- decreasing PCR amplicon size</li></ul> <ul style="list-style-type: none"><li>• Use larger PCR targets (e.g. &gt; 500 bp) or intron-spanning primers if possible</li><li>• Use protocol at Section 5.9 for DNase digestion in solution.</li></ul>

## **Problem: Suboptimal performance of RNA in downstream experiments**

<b>Possible cause</b>	<b>Suggestions</b>
<i>Carry-over of ethanol or salt</i>	<ul style="list-style-type: none"><li>• Do not let the flowthrough touch the column outlet after the second wash with <b>Wash Buffer II</b>. Be sure to centrifuge at the corresponding speed for the respective time in order to remove ethanolic <b>Wash Buffer II</b> completely.</li></ul>

## Problem: Suboptimal performance of RNA in downstream experiments, continued

Possible cause	Suggestions
<i>Carry-over of ethanol or salt, continued</i>	<ul style="list-style-type: none"><li>• Check if <b>Wash Buffer II</b> has been equilibrated to room temperature before use. Washing at lower temperatures lowers efficiency of salt removal by <b>Wash Buffer II</b>.</li></ul>
<i>Store isolated RNA properly</i>	<ul style="list-style-type: none"><li>• Eluted RNA should always be kept on ice for optimal stability since trace contaminations of omnipresent RNases will degrade the isolated RNA. For short-term storage, freeze at -20°C; for long-term storage, freeze at -80°C.</li></ul>

## 6.6. Related products

A full range of molecular biology reagents can be found on the GE Healthcare website and in the catalog. If you need further information, GE technical services are happy to assist.

<http://www.gehealthcare.com/illustra>

Application	Product Number	Product	Pack sizes
Kits containing ready-to-use mix for RT-PCR amplification	27-9266-01	illustra Ready-To-Go™ RT-PCR Beads (0.5 ml tubes)	100 reactions
	27-9267-01	illustra Ready-To-Go RT-PCR Beads (0.2 ml tubes)	96 reactions

<b>Application</b>	<b>Product Number</b>	<b>Product</b>	<b>Pack sizes</b>
<b>Kits containing ready-to-use mix for RT-PCR amplification, continued</b>	27-9557-02	illustra Ready-To-Go RT-PCR Beads (0.2 ml hinged tube with cap)	96 reactions
<b>1st Strand cDNA Synthesis</b>	27-9261-01	First-Strand cDNA Synthesis Kit	55 reactions
	27-9264-01	Ready-To-Go You-Prime First-Strand Beads	50 reactions
	27-9263-01	Ready-To-Go T-Primed First-Strand Kit	50 reactions

## 7. References

1. Web site for Agilent Bioanalyzer at <http://www.home.agilent.com>.

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## 1. Homogenization and Lysis

### 1.1. Tissue homogenization and lysis using a mortar & pestle

- ⊕ Liquid N<sub>2</sub> into mortar & pestle
- ⊖ Up to 30 mg tissue
- ⊕ 350 µl Lysis Solution and 3.5 µl β-mercaptoethanol
- ⊕ Until no tissue pieces visible

### 1.2. Tissue homogenization and lysis using a rotor-stator

- ⊕ 350 µl Lysis Solution and 3.5 µl β-mercaptoethanol
- ⊖ Up to 30 mg tissue until no tissue pieces visible

### 1.3. Homogenization and lysis of cultured cells

- Count up to  $5 \times 10^6$  cells
- ⌚ 1 min 5000 × g; discard supernatant
- ⊕ 500 µl PBS
- ⌚ 1 min 5000 × g; discard supernatant
- ⊕ 350 µl Lysis Solution and 3.5 µl β-mercaptoethanol; resuspend the pellet

## 2. Filtration

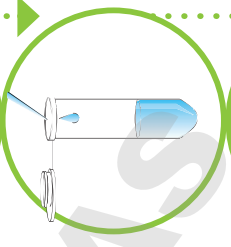
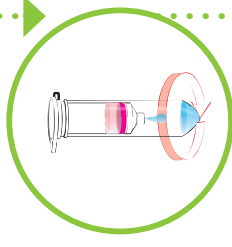
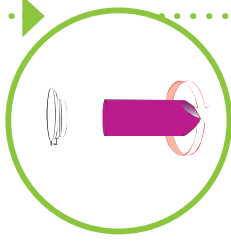
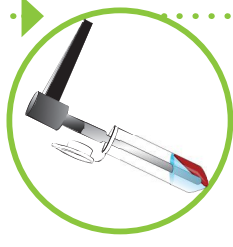
- Transfer sample to RNAspin Mini Filter inside collection tube
- ⌚ 1 min 11 000 × g; discard RNAspin Mini Filter

## 3. Adjust RNA binding condition

- ⊕ 350 µl ethanol; vortex twice for 5 s each

## 4. RNA binding

- Transfer sample to RNAspin Mini Column inside collection tube
- ⌚ 30 s 8000 × g



- Transfer column to a new collection tube







## 5. Desalting

- + 350  $\mu$ l  Desalting Buffer
-  1 min 11 000 x g; discard the flowthrough

## 6. DNA digestion

- +  95  $\mu$ l DNase I reaction mixture (10%  DNase I and 90%  DNase I Reaction Buffer)
- 15 min room temperature

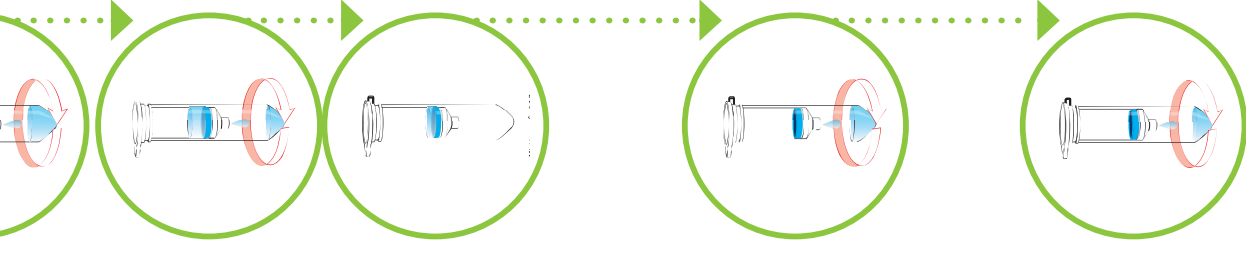
## 7. Wash and dry

- +  200  $\mu$ l Wash Buffer I
-  1 min 11 000 x g; transfer column to a new collection tube
- +  600  $\mu$ l Wash Buffer II
-  1 min 11 000 x g; discard the flowthrough
- +  250  $\mu$ l Wash Buffer II
-  2 min 11 000 x g
- Transfer the column to a new RNase-free 1.5 ml Microcentrifuge Tube

## 8. Elution

- +  100  $\mu$ l RNase-free H<sub>2</sub>O
-  1 min 11 000 x g

- + : Add
-  : Homogenize
-  : Spin
-  : Incubate



## Quick Reference Protocol Card

illustra™ RNAspin Mini RNA Isolation Kit

25-0500-70 (20 purifications)

25-0500-71 (50 purifications)

25-0500-72 (250 purifications)

### **Standard protocol for total RNA purification from cultured cells and tissue.**

- 1<sup>st</sup> time users of RNAspin kit should follow the detailed protocol in section 5.
- The quick reference protocol is for experienced users only.
- Ensure no precipitate present in Lysis Solution.
- Ensure ethanol added to Wash Buffer II.

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### Restrictions for product use

The components of the **illustra™ RNAspin Mini RNA Isolation Kit** have been designed, developed and sold for **research purposes only**. They are suitable for **in vitro use only**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking). It is the responsibility of the user to verify the use of **illustra RNAspin Mini RNA Isolation Kit** for a specific application range.

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