ISOLATE RNA Kits

Product Manual

ISOLATE RNA Mini Kit
ISOLATE Plant RNA Mini Kit

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1. KIT CONTENTS

REAGENT	10 MINIPREPS	50 MINIPREPS	250 MINIPREPS
Lysis Buffer R	6ml	30ml	125ml
Wash Buffer AR	3ml	15ml	70ml
Wash Buffer BR	2ml	8ml	40ml
RNase-free Water	1.5ml	6ml	2 x 15ml
Spin Column R1	10	50	5 x 50
Spin Column R2	10	50	5 x 50
Collection Tube	50	5 x 50	25 x 50
Elution Tube	10	50	5 x 50
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2. DESCRIPTION

ISOLATE RNA Mini Kit is specially designed for the fast and efficient isolation of extremely pure total RNA from a variety of samples. The kit is compatible with cultured cells, tissues and bacterial cells. The isolated RNA is ready for downstream applications such as reverse transcription, real-time PCR and RNA protection assays.

The protocol is easy to follow on a step-by-step basis. The cells are lysed with an optimized Lysis Buffer, which simultaneously inactivates RNases. The lysate is then applied to a Spin Column to selectively remove genomic DNA. There is no need to perform a separate DNase digestion step. The lysis buffer is optimized to efficiently lyse the cells in a chaotropic lysis buffer. The lysis buffer also inactivates RNases, thus protecting the released RNA. The RNA is then bound to a silica membrane. Subsequent wash steps remove the remaining cell debris. Pure RNA is eluted in the final step with RNase-free water.

Features

- High purity RNA
- Rapid protocol: 15-20 minutes
- Clear, easy to follow instructions

Applications

Isolation of RNA from:

- Animal tissue
- Eukaryotic cells
- Bacterial cells

3. STORAGE

The ISOLATE RNA Mini Kit should be stored dry at room temperature. Under these conditions, the kit is stable for 12 months.

4. SAFETY INFORMATION

Always wear gloves and a suitable lab coat when handling the reagents of this kit. For detailed information, refer to the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

Starting material

- Animal tissue (up to 20mg)
- Eukaryotic cells (up to 5 x 10^6 cells)
- Bacterial cells (up to 1 x 10^9 cells)

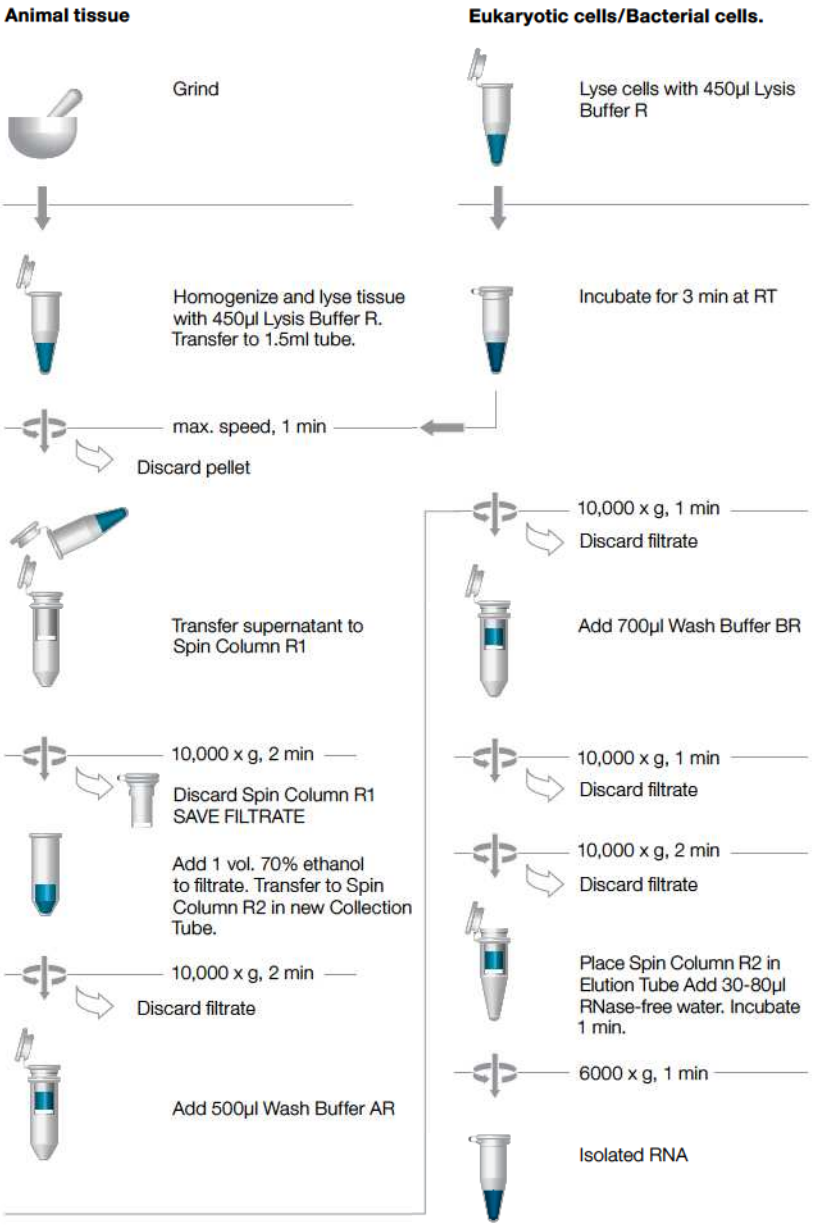
Time required

15-20 minutes

Binding capacity

Approximately 100µg RNA

Total RNA isolation



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

- Suitable container to hold sample
- Mortar and pestle and liquid N_2 or rotor stator homogenizer
- Microcentrifuge with rotor for 1.5ml and 2.0ml tubes
- Shaking platform
- 70% and 96-100% ethanol
- TE Buffer
- ddH_2O

7. PROTOCOLS

7.1 Total RNA isolation from animal tissue

Before you start:

- Before using for the first time, add 96-100% ethanol to the Wash Buffers AR and BR as indicated on the bottles and mix.
- If any of the buffers form precipitates upon storage, re-dissolve by gently warming. Cool to room temperature before use.
- If using frozen tissue, do not allow tissue to thaw during weighing or before the addition of Lysis Buffer R. Once homogenized in the Lysis Buffer, the sample can be stored at -20°C for several months.
- For information on how to work with RNA, read Hints and Tips on page 19.

1. Homogenize and lyse up to 20mg of tissue sample using liquid nitrogen or a rotor-stator homogenizer.

Using liquid nitrogen

1.1. Grind the sample to a fine powder using a mortar and pestle in the presence of liquid nitrogen. Take care that the sample does not thaw during or after grinding.

1.2. Transfer the sample to a 1.5ml microcentrifuge tube (not supplied).

1.3. Immediately add 450µl Lysis Buffer R and homogenize the sample. Proceed to next step. The sample can also be stored at this step at -20°C.

Using a rotor-stator homogenizer

1.1 Transfer the sample to a suitable container.

1.2 Add 450µl Lysis Buffer R and homogenize the sample.

1.3 Transfer the sample to a 1.5ml microcentrifuge tube (not supplied). Proceed to next step. The sample can also be stored at this step at -20°C.

2. **Centrifuge the lysate at maximum speed for 1 minute. Place Spin Column R1 into a 2ml Collection Tube. Carefully transfer the supernatant from the sample to Spin Column R1. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard Spin Column R1 and SAVE THE FILTRATE (for optional DNase digestion see Hints and tips, Page 20).**
   Note: Ensure that there is no lysate remaining on Spin Column R1. If required, centrifuge Spin Column R1 again until all liquid has passed through the membrane.

3. **Add 1 volume (usually 400µl) of 70% ethanol to the filtrate and mix well by pipetting. Transfer immediately to Spin Column R2 placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the filtrate and place Spin Column R2 in a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on Spin Column R2. If required, centrifuge Spin Column R2 again until all liquid has passed through the membrane.

4. **Add 500µl Wash Buffer AR to Spin Column R2. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 in a new Collection Tube.**
   Note: Ensure that ethanol has been added to Wash Buffer AR according to the instructions on the bottle.

5. **Add 700µl Wash Buffer BR to the Spin Column 2. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 in a new Collection Tube.**
   Note: Ensure that ethanol has been added to Wash Buffer BR according to the instructions on the bottle.

6. **Centrifuge at 10,000 x g (12,000rpm) for 2 minutes to remove all traces of ethanol. Discard the filtrate and place Spin Column R2 in an Elution Tube.**

7. **Add 30-80µl RNase-free water directly to Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the RNA.**
   Note: Use a lower volume of RNase-free water if a high concentration of RNA is required. Increasing the volume of water will increase the yield but decrease the concentration of RNA. Optionally, perform a second elution step to increase the yield.

8. **The isolated RNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

7.2 Total RNA isolation from eukaryotic cells

Before you start:

- Before using for the first time, add 96-100% ethanol to the Wash Buffers AR and BR as indicated on the bottles and mix.
- If any of the buffers form precipitates upon storage, re-dissolve by gently warming. Cool to room temperature before use.
- Cells grown in cell culture vessels can be lysed directly in the vessel or trypsinized to detach from the vessel. Once homogenized in the Lysis Buffer, the sample can be stored at -20°C for several months.
- For information on how to work with RNA, read Hints and Tips on page 19.

1. **Harvest up to a maximum of 5 x 10^6 cells.**

Cells grown in suspension

- Centrifuge appropriate number of cells at 300 x g for 5 minutes. Remove all supernatant by aspiration taking care not to disturb the pellet.

Cells grown in a monolayer

- Remove the cell culture medium completely by aspiration. Incomplete removal of the medium will inhibit lysis of the cells and compromise the efficiency of RNA extraction. Proceed directly to step 2.

2. **Add 450µl Lysis Buffer R to the sample. Resuspend the sample completely by pipetting up and down a few times. Incubate the sample for 3 minutes at room temperature.**
   Note: No cell clumps should be visible after the lysis step to maximize RNA yield.

3. **Place Spin Column R1 into a 2ml Collection Tube. Carefully transfer the supernatant from the sample to Spin Column R1. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard Spin Column R1 and SAVE THE FILTRATE (for optional DNase digestion see Hints and tips, Page 20).**
   Note: Ensure that there is no lysate remaining on Spin Column R1. If required, centrifuge the Spin Column again until all liquid has passed through the membrane.

4. **Add 1 volume of 70% ethanol to the filtrate and mix well by pipetting. Transfer immediately to Spin Column R2 placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the filtrate and place Spin Column R2 in a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on Spin Column R2. If required, centrifuge the

Spin Column again until all liquid has passed through the membrane.

5. **Add 500µl Wash Buffer AR to Spin Column R2. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 in a new Collection Tube.**
   Note: Ensure that ethanol has been added to Wash Buffer AR according to the instructions on the bottle.

6. **Add 700µl Wash Buffer BR to Spin Column R2. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 in a new Collection Tube.**
   Note: Ensure that ethanol has been added to Wash Buffer BR according to the instructions on the bottle.

7. **Centrifuge at 10,000 x g (12,000rpm) for 2 minutes to remove all traces of ethanol. Discard the filtrate and place Spin Column R2 in an Elution Tube.**

8. **Add 30-80µl RNase-free water directly to Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the RNA.**
   Note: Use a lower volume of RNase-free water if a high concentration of RNA is required. Increasing the volume of water will increase the yield but decrease the concentration of RNA. Optionally, perform a second elution step to increase the yield.

9. **The isolated RNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

7.3 Total RNA isolation from bacterial cells

Before you start:

- Before using for the first time, add 96-100% ethanol to the Wash Buffers AR and BR as indicated on the bottles and mix.
- If any of the buffers form precipitates upon storage, re-dissolve by gently warming. Cool to room temperature before use.
- To maximize lysis efficiency and yield of total RNA, harvest cells in the mid-logarithmic phase.
- This protocol describes lysis of bacterial cells using lysozyme. Other methods, such as enzymatic lysis using Proteinase K or mechanical disruption, can also be used.
- For information on how to work with RNA, read Hints and Tips on page 19.

1. **Centrifuge bacterial cells for 4 minutes at 5000 x g. Discard the supernatant by aspiration.**
   Note: Up to 1 x 10^9 cells can be processed at a time. Remove the culture medium as much as possible without disturbing the pellet. Incomplete removal may compromise the

efficiency of RNA isolation.

2. **Resuspend the cell pellet in 100µl TE Buffer (not supplied). Add 2-6µl lysozyme solution (not supplied). Pipette up and down for a few times until the solution becomes slightly viscous.**
   Note: For Gram ve bacteria: Add 2µl of 20mg/ml lysozyme solution.
   For Gram +ve bacteria: Add 6µl of 50mg/ml lysozyme.

3. **Add 450µl Lysis Buffer R and vortex vigorously. Incubate for 3 minutes at room temperature.**
   Note: To maximize total RNA yield, ensure that no cell clumps are visible.

4. **Transfer the sample to Spin Column R1 placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard Spin Column and SAVE THE FILTRATE (for optional DNase digestion see Hints and tips, Page 20).**
   Note: Ensure that there is no lysate remaining on Spin Column R1. If required, centrifuge Spin Column R1 again until all liquid has passed through the membrane.

5. **Add 1 volume of 70% ethanol to the filtrate and mix well by pipetting.**

6. **Transfer 650µl of the sample to Spin Column R2 placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 in a new Collection Tube. Transfer the remaining sample from step 5 to the same Spin Column R2 and centrifuge again at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 into a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on Spin Column R2 If required, centrifuge Spin Column R2 again until all liquid has passed through the membrane.

7. **Add 500µl Wash Buffer AR and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 into a new Collection Tube.**

8. **Add 700µl Wash Buffer BR and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column R2 into a new Collection Tube.**

9. **Centrifuge at 10,000 x g (12,000rpm) for 2 minutes to remove all traces of ethanol. Discard the filtrate and place Spin Column R2 in an Elution Tube.**

10. **Add 30-80µl RNase-free water directly to Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the RNA.**
    Note: Use a lower volume of RNase-free water if a high concentration of RNA is required. Increasing the volume of water will increase the yield but decrease the concentration of RNA. Optionally, perform a second elution step to increase the yield.

11. **The isolated RNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

UBLESHOOTING GUIDE

VATION	POSSIBLE CAUSE	RECOMMENDED VSOLUTION
d Spin Column	Insufficient disruption or homogenization	Reduce starting material.
	Insufficient centrifugation	Increase centrifugation speed and time.
A yield	Insufficient disruption or homogenization	Reduce amount of starting material.
	Incomplete elution	Incubate sample in Elution Tube with RNase-free water for up to 5 minutes and repeat elution step.
A concentration	High elution volume	Elute RNA with a lower volume. Do not use less than 20µl.
RNA degraded	Inappropriate handling and storing of starting material	Ensure proper handling and storage of samples. Ensure that all steps are followed quickly.
	RNase contamination	Ensure an RNase free working environment (see page 19). Discard any solutions contaminated with RNase during use.
DNA contamination	Incorrect lysis	Check protocol has been followed correctly
	Too much starting material	Reduce amount of starting material.
RNA does not perform well in downstream applications	Ethanol carryover during elution	Increase centrifugation time for ethanol removal step
	Salt carryover during elution	Ensure that Wash Buffers are at room temperature. Check both solutions for salt precipitates. Resuspend any visible precipitate by gentle warming.

ISOLATE Plant RNA Mini Kit

1. KIT CONTENTS

REAGENT	10 MINIPREPS	50 MINIPREPS	250 MINIPREPS
Lysis Buffer APR	6ml	30ml	125ml
Lysis Buffer BPR	6ml	30ml	125ml
Wash Buffer APR	3ml	15ml	70ml
Wash Buffer BPR	3ml	15ml	2 x 40ml
RNase-free Water	1.5ml	6ml	2 x 15ml
Spin Column PR1	10	50	5 x 50
Spin Column PR2	10	50	5 x 50
Collection Tube	60	6 x 50	30 x 50
Elution Tube	10	50	5 x 50
Product Manual	1	1	1
Bench Protocol Sheet	1	1	1

2. DESCRIPTION

ISOLATE Plant RNA Mini Kit is specially designed for the fast and efficient isolation of extremely pure total RNA from a variety of plant tissue samples. The isolated RNA is ready for downstream applications such as reverse transcription, real-time PCR and RNA protection assays.

The protocol is easy to follow on a step-by-step basis. Two lysis buffers are provided. In most cases, Lysis Buffer APR is recommended. In cases where yield is poor with Lysis Buffer APR, Lysis Buffer BPR should be used. Once the cells are lysed, the lysate is applied to a Spin Column to selectively remove genomic DNA. There is no need to perform a separate DNase digestion step. The lysis buffer is optimized to efficiently lyse the cells by incubation in a chaotropic lysis buffer. The lysis buffer also inactivates RNases, thus protecting the released RNA. The RNA is then bound to a silica membrane. Subsequent wash steps remove the remaining cell debris. Pure RNA is eluted in the final step with RNase-free water.

Features

- Rapid protocol: 30 minutes after homogenization
- High purity RNA
- Clear, easy to follow instructions

Applications

Isolation of RNA from:

- Fresh or frozen plant material

3. STORAGE

The ISOLATE Plant RNA Mini Kit should be stored dry at room temperature. Under these conditions, the kit is stable for 12 months.

4. SAFETY INFORMATION

Always wear gloves and a suitable lab coat when handling the reagents of this kit. For detailed information, refer to the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

Starting material

Plant tissue (up to 100mg)

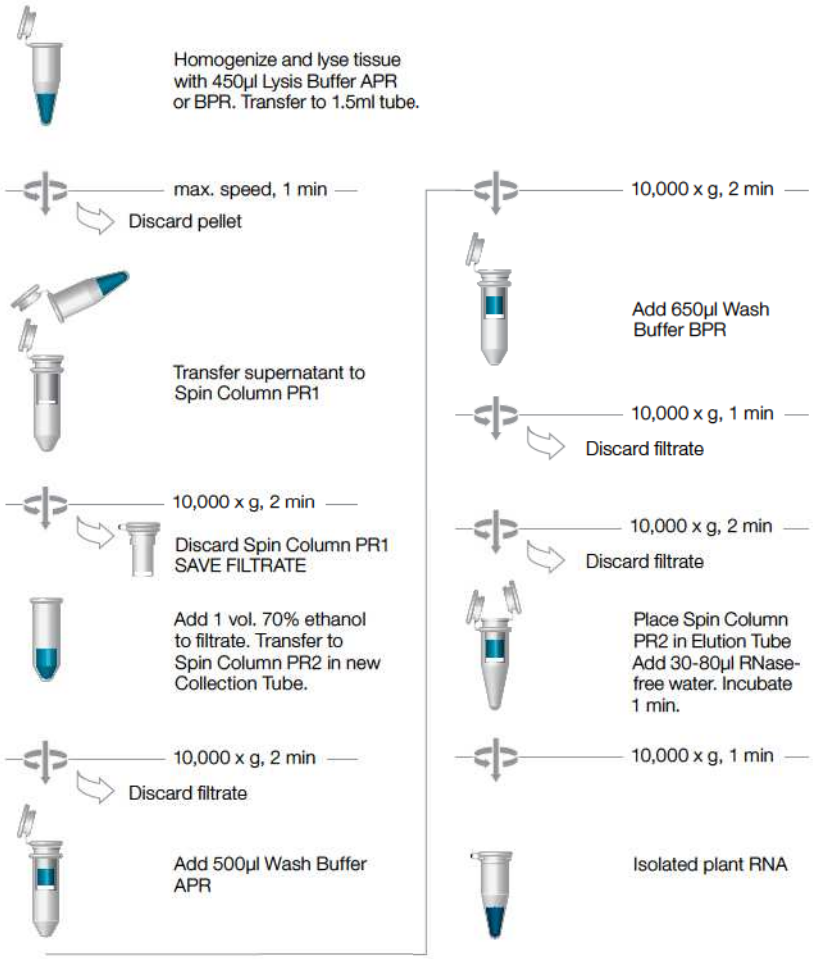
Binding capacity

Approximately 100µg RNA

Time required

30 minutes after homogenization

Total RNA isolation from plant tissue



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY THE USER

- Suitable container to hold sample
- Mortar and pestle and liquid N_2 or rotor stator homogenizer
- Microcentrifuge with rotor for 1.5ml and 2.0ml tubes
- Shaking platform
- 70% and 96-100% ethanol

7. PROTOCOL

7.1 Total RNA isolation from plant tissue samples

Before you start:

- Before using for the first time, add 96-100% ethanol to the Wash Buffers AR and BR as indicated on the bottles and mix.
- Avoid freezing and thawing of starting material.
- If any of the buffers form precipitates upon storage, re-dissolve by gently warming. Cool to room temperature before use.
- For information on how to work with RNA, read Hints and Tips on page 19.

1. Homogenize and lyse up to 100mg of fresh or frozen plant tissue sample using liquid nitrogen or a rotor-stator homogenizer.

Using liquid nitrogen

1.1. Grind the sample to a fine powder using a mortar and pestle in the presence of liquid nitrogen. Take care that the sample does not thaw during or after grinding.

1.2. Transfer the sample to a 1.5ml microcentrifuge tube (not supplied).

1.3. Immediately add 450µl Lysis Buffer APR or BPR and homogenize the sample. Proceed to next step. The sample can also be stored at this step at -20°C.
Note: Most plant material can be lysed using Lysis Buffer APR and is the recommended buffer for all applications. In case of low RNA yield, repeat the experiment with Lysis Buffer BPR.

Using a rotor-stator homogenizer

1.1. Transfer the sample to a suitable container.

1.2. Add 450µl Lysis Buffer APR or BPR and homogenize the sample.
Note: Most plant material can be lysed using Lysis Buffer APR and is the recommended buffer for all applications. In case of low RNA yield, repeat the experiment with Lysis Buffer BPR.

1.3. Transfer the sample to a 1.5ml microcentrifuge tube (not supplied). Proceed to next step. The sample can also be stored at this step at -20°C.

2. **Centrifuge the lysate at maximum speed for 1 minute. Place Spin Column PR1 in a 2ml Collection Tube. Carefully transfer the supernatant from the sample to Spin Column PR1. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard Spin Column PR1 and SAVE THE FILTRATE (for optional DNase digestion see Hints and tips, Page 20).**
   Note: Ensure that there is no lysate remaining on Spin Column PR1 filter. If required, centrifuge Spin Column PR1 again until all liquid has passed through the membrane.

3. **Add 1 volume (usually 400µl) of 70% ethanol to the filtrate and mix well by pipetting. Transfer immediately to a Spin Column PR2 placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the filtrate and place Spin Column PR2 in a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on Spin Column PR2. If required, centrifuge Spin Column PR2 again until all liquid has passed through the membrane.

4. **Add 500µl Wash Buffer APR to Spin Column PR2. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column PR2 in a new Collection Tube.**
   Note: Ensure that ethanol has been added to Wash Buffer AR according to the instructions on the bottle.

5. **Add 650µl Wash Buffer BPR to the Spin Column PR2. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and place Spin Column PR2 in a new Collection Tube.**
   Note: Ensure that ethanol has been added to Wash Buffer BR according to the instructions on the bottle.

6. **Centrifuge at 10,000 x g (12,000rpm) for 2 minutes to remove all traces of ethanol. Discard the filtrate and place Spin Column PR2 in an Elution Tube.**

7. **Add 30-80µl RNase-free water directly to the Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the RNA.**
   Note: Use a lower volume of RNase-free water if a high concentration of RNA is required. Increasing the volume of water will increase the yield but decrease the concentration of RNA. Optionally, perform a second elution step to increase the yield.

8. **The isolated RNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

8. TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION
Clogged Spin Column	Insufficient disruption or homogenization	Reduce starting material.
	Insufficient centrifugation	Increase centrifugation speed and time.
Low RNA yield	Insufficient disruption or homogenization	Reduce amount of starting material.
	Incomplete elution	Incubate sample in Elution Tube with RNase-free water for up to 5 minutes and repeat elution step.
Low RNA concentration	High elution volume	Elute RNA with a lower volume. Do not use less than 20µl.
	Inappropriate handling and storing of starting material	Ensure proper handling and storage of samples. Ensure that all steps are followed quickly.
RNA degraded	RNase contamination	Ensure an RNase free working environment (see page 10). Discard any solutions contaminated with RNase during use.
DNA contamination	Incorrect lysis	Check protocol has been followed correctly
	Too much starting material	Reduce amount of starting material.
RNA does not perform well in downstream applications	Ethanol carryover during elution	Increase centrifugation time for ethanol removal step
	Salt carryover during elution	Ensure that Wash Buffers are at room temperature. Check both solutions for salt precipitates. Resuspend any visible precipitate by gentle warming.

A. HINTS AND TIPS

1. Working with RNA

An RNase free environment is essential when working with RNA samples. In the laboratory, obtaining full length, high quality RNA often proves to be a daunting task. There are two main reasons for RNA degradation during RNA analysis. Firstly, RNA, by its very structure, is inherently weaker than DNA. RNA is made up of ribose units, which have a highly reactive hydroxyl group on C2 that takes part in RNA-mediated enzymatic events. This makes RNA more chemically labile than DNA. RNA is also more prone to heat degradation than DNA. Secondly, enzymes that degrade RNA, ribonucleases (RNases) are so ubiquitous and hardy that getting rid of them often proves to be nearly impossible. For example, autoclaving a solution containing bacteria will destroy the bacterial cells, but not the RNases released from the cells.

2. Sources of RNase

- Skin: The presence of RNases on human skin surfaces has been well documented. RNase contamination through this source is very easy to acquire and spread if tubes, pipette tips, bench tops, etc. are touched with bare hands.
- Dust: Dust particles floating in the air often harbor bacteria or mold. The RNases from these microorganisms get deposited wherever the dust settles. This includes lab equipment, open bottles, etc.
- Reagents: If the reagents used for RNA analysis are not certified to be RNase free, there is a good chance that some of the contamination will come from this source. Reagents can also become contaminated in the lab itself if proper care is not taken.
- Samples: RNase contamination can come from the samples themselves as tissues and cells contain endogenous RNases.

3. How to maintain an RNase-free environment

- Gloves: Always wear sterile gloves before handling any thing that is going to be used for RNA analysis. It is however important to remember that once the gloves have touched equipment in the lab such as centrifuges, pipettes and door handles, they are no longer RNase-free.
- Disposable plasticware: Disposable plasticware greatly reduce the possibility of contaminating your samples. In the event of a contamination, they also minimize the spread of the contamination. The use of disposable tips, tubes, etc. is therefore highly recommended.
- Good quality reagents: Always ensure that all reagents and chemical purchased commercially are guaranteed to be RNase free. Testing each batch before use may be a prudent step.
- DEPC-treated water: Use DEPC-treated water instead of regular PCR grade water. DEPC inactivates RNase by histidine modification of the bases. If DEPC-treated water is made in-house, always remember to autoclave before use to degrade the DEPC.
- RNase inhibitors: The use of RNase inhibitors is highly recommended with samples containing endogenous RNase. Most RNase inhibitors are suitable for use in any application where RNases are a potential problem.
- Decontamination techniques: Heat proof glassware can be baked at 180°C for several hours to inactive RNases. Polycarbonate or polystyrene materials can be decontaminated by soaking in 3% hydrogen peroxide for 15 minutes, followed by thorough rinsing with RNase-free water.
- Correct storage of RNA is also very important to avoid RNA degradation. In the short term, RNA may be stored in RNase-free H_2O or TE buffer at -80°C for 1 year without degradation. For long term storage RNA samples may be stored as ethanol precipitates at -20°C. However, when dissolved in ethanol, RNA is not dispersed evenly in the solution and cannot be used directly in quantitative experiments. Instead, precipitates should be pelleted and redissolved in an aqueous buffer before pipetting.

4. Determination of RNA yield, purity and integrity

The yield of total RNA may be determined spectrophotometrically at 260nm, whereby 1 unit of absorbance (A_{260}) = 40µg of single stranded RNA/ml. The purity can also be determined spectrophotometrically from the ratio of the relative absorbances at 260 and 280nm. Good quality RNA will have a A_{260}/A_{280} ratio in the range of 1.7 to 2.1.

The most common procedure for determining RNA integrity is running 2-4µg of a total RNA sample on an agarose denaturing gel. The RNA may be visualized by EtBr staining, which reveals the ribosomal RNA bands. These bands can vary depending on the organism the RNA was extracted from (see table on page 21). In general, for good quality RNA the bands should be distinct, with no smearing underneath them and the 28S band (larger) should be approximately twice as intense as the 18S band.

5. Optional DNase Digestion

Generally, DNase digestion is not required since ISOLATE technology efficiently removes most of the DNA without DNase treatment. However, further DNA removal may be necessary for certain RNA applications that are sensitive to very small amounts of DNA (e.g., real-time RT-PCR analysis with a low-abundant target), or samples very rich in DNA such as spleen tissue.

DNase is added to the filtrate of the R1 column and incubated 15min at 20-30°C before the addition of ethanol 70%. Although the DNase will be removed by the next extraction steps, a final heat inactivation (10min at 75°C) can be performed on the final eluate, to remove any residual DNase activity.

6. Ribosomal RNA Sizes

SPECIES	rRNA	SIZE(kb)
Human	18S	1.9
	28S	5.0
Mouse	18S	1.9
	28S	4.0
Drosophila	18S	2.0
	28S	4.1
Tobacco Leaf	16S	1.5
	18S	1.9
	23S	2.9
	25S	3.7
Yeast *(S. cerevisiae)*	18S	2.0
	26S	3.8
E. coli	16S	1.5
	23S	2.9
Xenopus	18S	1.8
	28S	4.0
Worm *(C. elegans)*	18S	1.7
	28S	3.5

B. TECHNICAL SUPPORT

For technical assistance or more information on these products, please call us on:

	SIZE	CAT NO.
	reps	BIO-52042
ISOLATE RNA Mini Kit	50 Preps	BIO-52043
ISOLATE RNA Mini Kit	250 Preps	BIO-52044
ISOLATE Plant RNA Mini Kit	10 Preps	BIO-52039
ISOLATE Plant RNA Mini Kit	50 Preps	BIO-52040
ISOLATE Plant RNA Mini Kit	250 Preps	BIO-52041

D. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
Agarose	500g	BIO-41025
Crystal 10x TE Buffer	10 Pouches	BIO-37105
DEPC-treated Water	10 x 10ml	BIO-38030
10x MOPS-EDTA-NA Acetate Buffer	1 Litre	BIO-38027
Elite Human HEK293 Total RNA	100µg	BIO-38034
Elite Human HeLa Total RNA	100µg	BIO-38035
Elite Mouse NIH3T3 Total RNA	100µg	BIO-38036
RiboSafe RNase Inhibitor	2500 Units	BIO-65027
cDNA Synthesis Kit	30 Reactions	BIO-65025
SensiMix SYBR One-Step Kit	250 Reactions	QT245-02
SensiMix Probe One-Step Kit	250 Reactions	QT725-02

E. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace free of charge any product that does not conform to the specifications. This warranty limits Bioline's liability only to the replacement of the product.