Instruction Manual
Research Use Only

SPINeasy® RNA Kit for Yeast

For the isolation of RNA from Yeast sample

Size: 50 and 5 PREPS
Storage: 15-25 °C
Cat. No.: 116565050 (50 PREPS) /116565000 (5 PREPS)
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Table of Contents

1. Introduction to SPINeasy® RNA Kit for Yeast

SPINeasy® RNA Kit for Yeast is a high-performance RNA extraction kit which is developed based on silica-membrane spin-column technology. By combining with beads beating method which could homogenize the yeast cell walls within 40 sec, the SPINeasy® RNA Kit for Yeast is capable of isolating RNA from a wide range of yeast species. This kit employs a quick desalting technique to eliminate contaminating macromolecules while avoiding harmful chemical solvents, and spheroplasting to successfully release RNA through carbohydrate-containing cell walls, without the need of any enzymes. Up to 2×10^8 cells can be handled at once without any DNase treatment. The resulting RNA is of exceptional purity, free of proteins or DNA in eluted samples, and suitable for use in any downstream applications such as Northern blotting, RT-PCR, Next-Generation Sequencing, Microarray, hybridization, and other sensitive applications. This kit is highly recommended for obtaining total RNA from yeast in less than 30 min.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Sample	Yeast
Sample amount	up to 2×10^8 cells.
Observed yield	up to 220 µg (number of cells and processing dependent)
Elution volume	50–100 µL
Preparation time	30 min (6 preps)

2. Kit Components and User Supplied Materials

2.1 SPINeasy® RNA Kit for Yeast Component

Product	50 reactions (Cat. No.: 116565050)		5 reactions (Cat. No.: 116565000)	
	Package	Cat. No.	Package	Cat. No.
Equilibration buffer	24 mL	116554063	2.4 mL	116554003
Lysing Matrix YG	50 x 2.0 mL	116557051	5 x 2.0 mL	116557001
Proteinase K	1.2 mL	116558056	120 µL	116558006
Lysis Buffer LB	40 mL	116565051	4 mL	116565001
Binding Buffer BB	8 mL	116565052	800 µL	116565002
Wash Buffer Y2	9 mL	116565053	900 µL	116565003
RNase Free Water	10 mL	116556056	1.0 mL	116556006
DNA Removal Column	50 ea	116565054	5 ea	116565004
RNA Collection Tubes	50 ea x 2	116554061	10 ea	116554010
RNA Column	50 ea	116554060	5 ea	116565006
2 mL Tubes	50 ea	116565055	5 ea	116565005
2.0 mL Collection Tubes	50 ea x 2	116530059	10 ea	116530011
Elution tubes	50 ea	116547062	5 ea	116547012
Quick-start protocol	1 ea	-	1 ea	-
Instruction Manual	Available www.mpbio.com			
MSDS & CoA	Available www.mpbio.com			

2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24® 5G (Cat. No.116005500) or Vortex.
- Microcentrifuge capable of spinning at ≥ 15,000 *g*.
- Single-channel pipettors (2 µL–1000 µL) and DNase/RNAse-free certified filter tips.
- β-mercaptoethanol (β-ME) solution such as MP Biomedicals Cat No. 194834
- Isopropanol
- Ethanol (> 95%)

3. Storage and Kit Stability

The SPINeasy® RNA Kit for Yeast is guaranteed until the expiry date stated on the kit when stored at room temperature (15-25˚C). For extended storage or storage in dry condition (humidity < 40%), pls store the DNA Removal Column and RNA Column at 2-8˚C to maintain performance.

4. Important Consideration Before Use

- ❑ SPINeasy RNA Kit for Yeast requires the use of a centrifuge capable of generating at least **15,000 *g*** to obtain optimal results.
- ❑ Shake the **Lysis Buffer LB** before use.
- ❑ Add **2.0 mL** of isopropanol to the **Binding Buffer BB**.
- ❑ Add **90 mL** of ethanol to the **Wash Buffer Y2**.
- ❑ An RNase-free environment is essential to obtain intact RNA samples.
- Use proper microbiological, aseptic technique.
- The most prevalent sources of RNase contamination are hands and dust particles that contain bacteria and mould. When handling reagents and RNA samples, always use disposable gloves and change them periodically.
- Use the disposable plasticwares provided in the kit. The use of certified DNase/RNAse-free filter tips is highly recommended.
- Keep tubes closed whenever possible.
- Following extraction, keep purified RNA on ice for immediate use or store the samples at -80 ˚C.
- ❑ Gel electrophoresis: Soak electrophoresis tanks, trays, and combs in 0.5% SDS solution for 10 min, then rinse with RNase-free water, ethanol, and allow to dry. Make an electrophoresis buffer with RNase-free water. The addition of

mercaptoethanol (0.5 µL) to the loading dye before mixing with the RNA sample, as well as bleach solution (1%) to the melted agarose solution, may aid in RNA integrity preservation.

- ❑ Avoid unnecessary freeze-thaw cycles of purified RNA. Aliquots should be made consistent with downstream needs.
- ❑ Cells grown in liquid culture should be pelleted and all supernatants should be removed prior to sample disruption and homogenization.

5. Safety Precaution

Wear personal protective equipment (gloves, lab coat, and eye protection) to avoid skin or mucous membrane contact. For more information, please see the Material Safety Data Sheet at www.mpbio.com. Because the equilibration buffer is corrosive, it can cause skin burns and eye damage. When working with yeast cultures, practice caution. Spills on hands must be carefully cleansed because they might cause skin diseases. **Binding Buffer BB** contains salts that are chaotropic, which when coupled with bleach can generate highly reactive compounds. **Binding Buffer BB** includes flammable isopropanol and ethanol.

6. Protocol

1. Column preparation

- To ensure optimal performance, the DNA Removal Column and RNA Column are pre-treated before use. Add **200 µL Equilibration Buffer** into the DNA and RNA column.
- Wait for at least **1 min**, centrifuge for **10 sec** @ maximum speed and transfer the column into new **2.0 mL Collection tube** and **RNA Collection Tube** (provided).
- If necessary, the treated column can be stored at **4-8° C** for up to **10 days**.

2. Sample preparation and Homogenization

- Transfer the yeast cell culture (up to 2 x 10^8 yeast cells) to a 2.0 mL microcentrifuge tube. Centrifuge the cells for **3-5 min** at **15,000 *g*.** Discard the supernatant.
- Resuspend the cell pellet in **750 µL Lysis Buffer LB** supplemented with **20 µL Proteinase K** and **2% of ß-Mercaptoethanol (ß-Mer)** (not provided).
- Transfer all the mixture to the **Lysing Matrix YG tube**.

- Homogenize using vortex at **2500 rpm** for **20 min** or FastPrep at **6m/s** for **40 sec**, then centrifuge for **2 min** at **15,000 *g*** and transfer the supernatant to **2 mL Tube** (provided).

  Note: The performance of the RNA output (yield, purity, and RNA integrity) obtained using vortex is highly dependent on the model of vortex used. The condition stated above can be used as a starting point. The homogenization time and speed should be optimized by the user. Avoid using tape or handling the matrix tube with your hands, which can result in reduced homogenization efficiency, inconsistent results, and reduced yields.

3. **DNA Removal**

   - Add **160 µL Binding Buffer BB** to the supernatant **(630 µL)** from the above step, vortex for **30 sec**, then centrifuge for **2 min** at **15,000 *g***.
   - Transfer the clear supernatant **(650 µL)** to the pre-treated DNA Removal Column and centrifuge at **15,000 *g*** for **20 sec** to bind/remove the genomic DNA from the lysate. Discard the column.

4. **RNA Binding**

   - To the flowthrough from the above step, add **1 volume of ethanol**. For example, add **650 µL ethanol** to **650 µL supernatant**.
   - Load **700 µL** of the above mixture into the **RNA Column** and centrifuge for **20 sec** at **15,000 *g***.
   - Repeat until all the above mixture has been transferred to the RNA Column.

5. **RNA Washing**

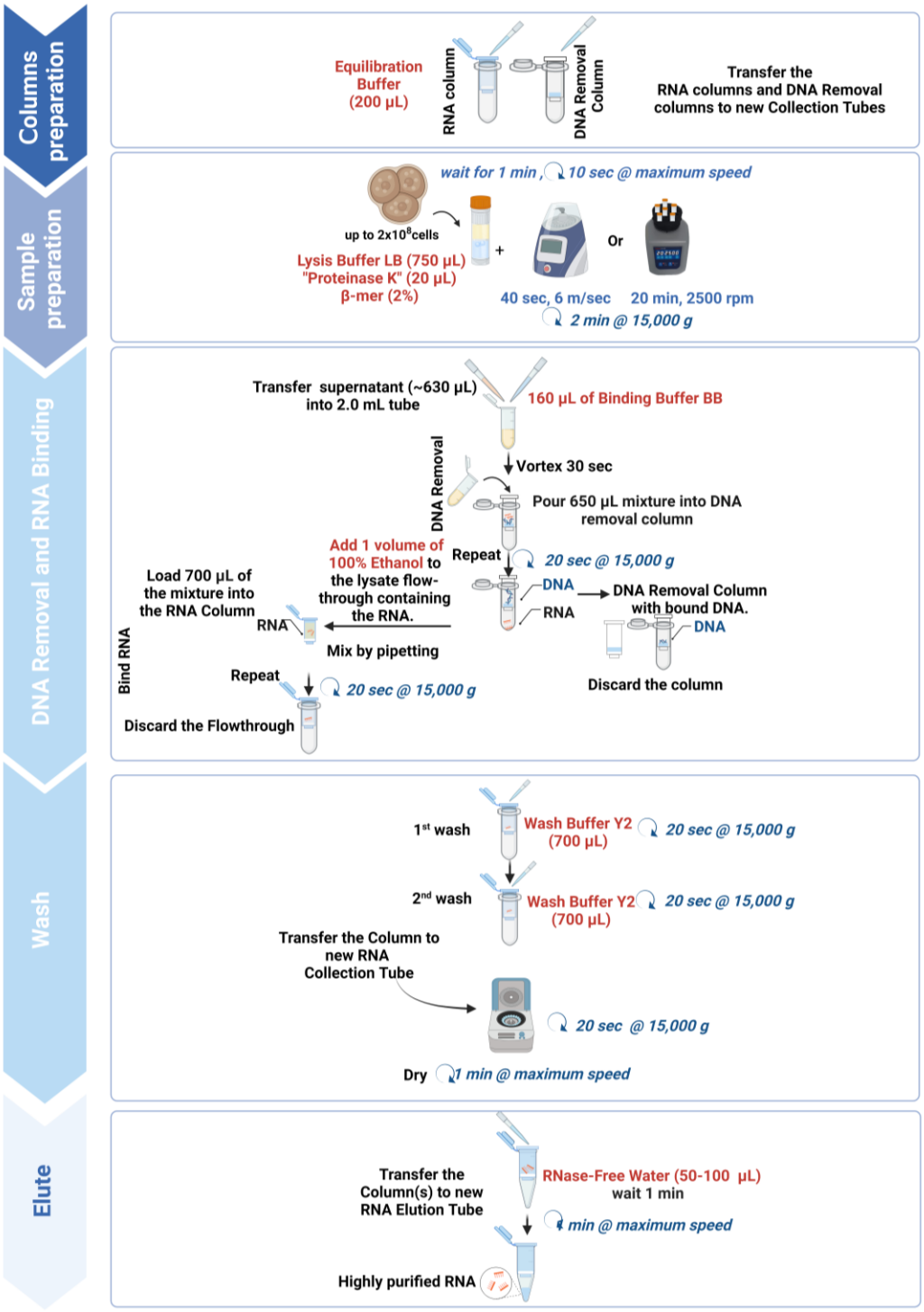
   - Add **700 µL Wash Buffer Y2** to the RNA Column and centrifuge for **20 sec** at **15,000 *g***.
   - Repeat the above wash again.
   - Transfer the RNA Column to a new Collection Tube and spin for **1 min** at maximum speed to remove any residual ethanol.

6. Elution

- Transfer the RNA Column to a new 1.5 mL Elution Tube. Add **100 µL RNAse Free Water** to the center of the column, wait for **1 min**, and then centrifuge at maximum speed for **1 min**.
- The RNA samples are now ready for downstream processing.

Note: Eluting with 100 µL RNase Free Water will maximize nucleic acid yield. For a more concentrated sample, a minimum of 50 µL RNase Free Water can be used.
Note: The nucleic acid concentration of the sample is calculated by its UV absorbance at 260 nm where an absorbance of 1 (1 cm path length) is equivalent to 50 µL RNA/mL. Contamination with DNA/RNA, protein, salt, ethanol, or other non-nucleic acid contaminants contributes to the total absorption at 260 nm and therefore leads to an overestimation of the real RNA concentration. When measured using a UV spectroscopy, a ratio of A260/A280 between 1.80–1.90 and A260/A230 >1.8 indicates DNA. A260/A280 and A260/230 ratio above 2.0 indicate RNA. Conversely, an A260/A280 ratio below 1.8 indicates protein contamination. Additionally, a low A260 / A230 ratio indicates the presence of humic acids as well as proteins, saccharides, ethanol, salt, and other contaminants which may inhibit subsequent enzymatic reactions.

7. Flow Chart


Columns preparation
Sample preparation
DNA Removal and RNA Binding
Wash
Elute
Equilibration Buffer (200 µL)
RNA column
DNA Removal Column
Transfer the RNA columns and DNA Removal columns to new Collection Tubes
wait for 1 min , 10 sec @ maximum speed
up to 2x10^8 cells
Lysis Buffer LB (750 µL)
"Proteinase K" (20 µL)
β-mer (2%)
Or
40 sec, 6 m/sec
20 min, 2500 rpm
2 min @ 15,000 g
Transfer supernatant (~630 µL) into 2.0 mL tube
160 µL of Binding Buffer BB
Vortex 30 sec
DNA Removal
Pour 650 µL mixture into DNA removal column
Repeat
20 sec @ 15,000 g
DNA
RNA
DNA Removal Column with bound DNA.
DNA
Discard the column
Add 1 volume of 100% Ethanol to the lysate flow-through containing the RNA.
Load 700 µL of the mixture into the RNA Column
RNA
Mix by pipetting
Bind RNA
Repeat
20 sec @ 15,000 g
Discard the Flowthrough
1st wash
Wash Buffer Y2 (700 µL)
20 sec @ 15,000 g
2nd wash
Wash Buffer Y2 (700 µL)
20 sec @ 15,000 g
Transfer the Column to new RNA Collection Tube
20 sec @ 15,000 g
Dry
1 min @ maximum speed
Transfer the Column(s) to new RNA Elution Tube
RNase-Free Water (50-100 µL)
wait 1 min
1 min @ maximum speed
Highly purified RNA

8. Data

1. **Figure 1.** SPINeasy® RNA Kit for Yeast can generate high quality RNA from 2 x 10^8 yeast cells.

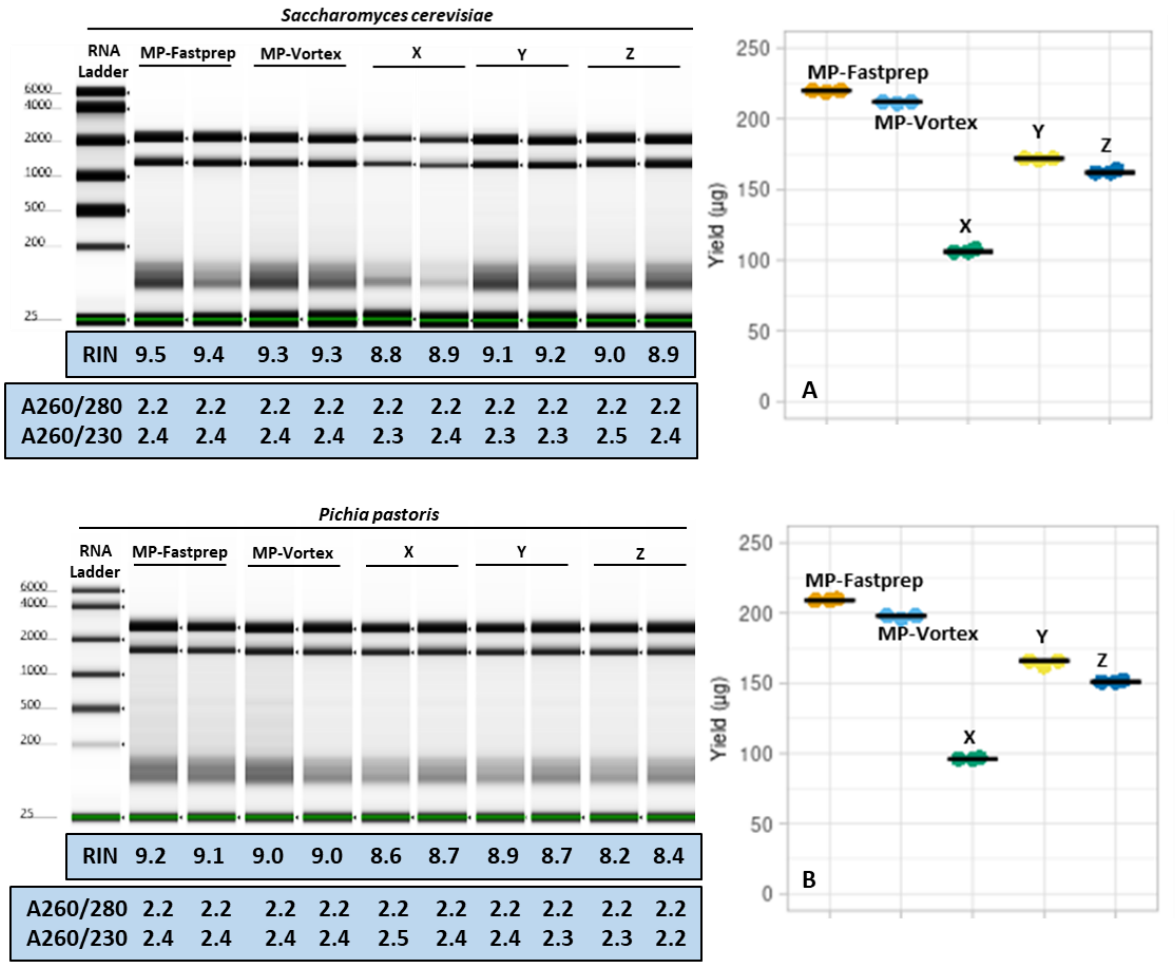


Figure 1. Total RNA from Yeast (*S. cerevisiae*) (figure 1A) **and *P. pastoris*)** (figure 1B) **was purified using the MP SPINeasy® RNA Kit for Yeast.** Extraction was done with both FastPrep® and vortex method. The extracted samples were compared with competitor X, Y and Z. The aliquots were run on an Agilent® Bioanalyzer 4150 using the RNA Screen tape. RIN values and O.D. ratios confirm the overall integrity and purity of the RNA.

2. **Figure 2:** SPINeasy® RNA Kit for Yeast can isolate high-quality RNA that is compatible with RT-qPCR.

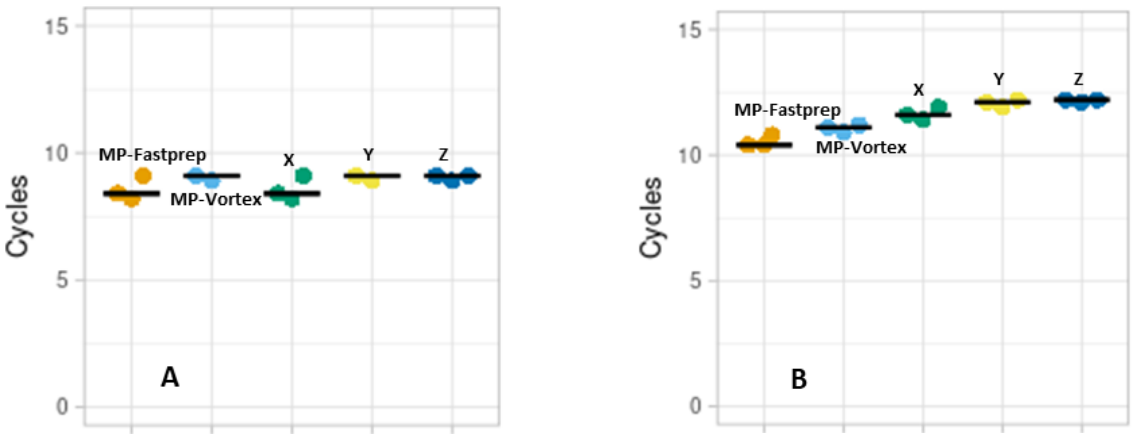


Figure 2: **High quality qPCR curves indicate accurate detection and quantitation of targets.** To demonstrate compatibility with downstream applications, samples were subsequently used for RT-PCR (+/RT) for detection of Yeast RNA species using iTaq™ Universal SYBR® Green Supermix (BioRad 1725121). Purified RNA from *S. cerevisiae* (Figure 2A) and *P. pastoris* (Figure 2B) was diluted to produce a five-log range of input template concentrations. Primers targeting Actins were used for RT-qPCR assays, assembled as directed by iTaq™ Universal SYBR® Green One-Step Kit RT-qPCR reagents (BioRad 1725150) and cycled on a QuantStudio3 (Applied Biosystems, by ThermoScientific)

3. **Table 1:** Expected RNA yield using SPINeasy® Kit for Yeast.

Number of Yeast	Expected Yield (RNA)
1 x 10^6	50 - 80 µg
1 x 10^7	90 - 110 µg
1 x 10^8	130 - 180 µg
2 x 10^8	170 - 220 µg

Table 1: SPINeasy® RNA Kit is validated on two types of yeast species (*S. cerevisiae* and *P. pastoris*), but it can also be used for other species of yeast. Handling of more than 2 x 10^8 yeast cells is possible, however it may require further DNase I treatment (to be standardized according to the manufacturer's guidelines).

4. **Table 2:** Competitor comparative data

Yeast	*S. cerevisiae*	*P. Pastoris*	Recommended cell numbers	Lysis method	DNA removal	Duration (Mins.)
No. of Cells	2 x 10^8 cells	2 x 10^8 cells	-	-	-	-
MP SPINeasy (#116565050)	220 µg	185 µg	2 x 10^8 cells	Bead beating-YG (Vortex/Fastprep)	gDNA column removal	15-30
Competitor X	81.7 µg	67.2 µg	1 x 10^7 cells	TissueLyser/ *Lyticase/Zymolase*	On-column *DNase* treatment/*DNase* treatment following purification	47-55
Competitor Y	110 µg	85.2 µg	4 x 10^8 cells	*Lyticase*	*DNase* treatment following purification	60-70
Competitor Z	98.4 µg	83.2 µg	5 x 10^7 cells	*Zymolase*/ Glass beads (bead mill)	gDNA column Removal & on-column *DNase* treatment	30-45

Table 2: For the competitor kits, **RNA** was isolated from the specified number of yeast cells and followed the recommendation for DNA contamination as per the manufacturer's protocol.

9. Troubleshooting

Problem	Possible Cause	Recommendation
Low nucleic acid Yield / reduced integrity	Suboptimal homogenization	The time and/or speed used for FastPrep homogenization can be increased. Yeast homogenization using FastPrep for 40 sec and 6 m/sec is standardized. Increasing/decreasing may result in low yield. Partially degraded RNA can be observed in agarose gel. But it may not have an effect on PCR/qPCR. Alternatively, vortex can be used but may require further optimization; the time and/or speed can be increased up to 3000 rpm/ 30 min instead of 2500 rpm/20 min.
	Nucleic acid still bound to the column	Wait for 10 min after addition of RNase-free water before centrifuging. The RNase-free water used for RNA elution may be preheated to 70°C.
	The sample have been stored/frozen before extraction	Fresh samples are preferred to obtain optimal yield and integrity.
	RNase contamination	RNases are ubiquitous and stable laboratory contaminants and can be potentially introduced to a sample during experiment. Ensure that all the plasticware used are *RNase*-Free. Add ß-mercaptoethanol during the homogenization step. Follow the instruction stated on page 5 “Important Consideration Before Use”.

Low A260/230 or A260/280 ratios	High salt concentration or due to binding buffer	Additional wash may be required using Wash Buffer Y2. Rebound the eluted RNA samples to the same column and re-elute after 1 min of incubation.
	Clogged column	Reduce the number of samples. This kit has the capacity to isolate cells up to 1 x 10^9 but may have DNA contamination which can be eliminated through DNase digestion.
DNA contamination in RNA sample	Sample has high DNA content	Samples up to 2 x 10^8 will not have RNA contamination. Samples above the standardized count will have to undergo DNase digestion.
Poor PCR Performance	High concentration of nucleic acid	The sample should be diluted. A large volume of nucleic acid sample inhibits PCR. Big amounts of RNA molecules in the constricted space of the reaction vessel have been shown to cause erroneous priming, exhaustion of magnesium ions, primer(s), dNTP(s), and hinder the passage of big Taq polymerase molecules. If PCR with an undiluted sample is necessary, check the enzyme specification and manufacturer's instructions, or select an alternate PCR enzyme with high strand displacement activity. If PCR can be performed on diluted samples, the amount of RNA to be used is individual to each PCR enzyme and may require user optimization. Genes with numerous copies in the genome, such as ribosomal genes, require significantly less RNA input. SPINeasy® RNA Kit for Yeast provided positive amplifications from a variety of samples using as much as >200 ng or as little as <0.20 ng of RNA per PCR of 20 µL employing inhibitor sensitive PCR enzymes.
	Suboptimal PCR condition.	Verify PCR reagents and protocol with positive control; adjustment on reaction/cycle conditions or primer selection may be necessary following manufacturer recommendation.

10. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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Australia
Tel: +61 2.8824.2100
Tel: +61 1800.249.998
Email: custserv.au@mpbio.com

Austria & Germany
Tel: 0800.426.67.337
Tel: 00800.7777.9999
Email: custserv.de@mpbio.com

Belgium
Tel: 00800.7777.9999
Email: custserv.be@mpbio.com

Canada
Tel: +1 800.854.0530
Email: custserv.ca@mpbio.com

China
Tel: +86 400.150.0680
Email: custserv.cn@mpbio.com

Europe
Tel: +33 3.88.67.54.25
Tel: +33 00800.7777.9999
Email: custserv.eur@mpbio.com

France
Tel: +33 3.88.67.54.25
Email: custserv.fr@mpbio.com

India
Tel: +91 22.27636921/22/25
Email: custserv.in@mpbio.com

Italy
Tel: 00800.7777.9999
Email: custserv.it@mpbio.com

Japan
Tel: +81 3.6667.0730
Email: custserv.jp@mpbio.com

Latin America
Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.la@mpbio.com

New Zealand
Tel: +64 9.912.2460
Email: custserv.nz@mpbio.com

North America
Tel: +1 800.854.0530
Tel: +1 440.337.1200
Email: custserv.na@mpbio.com

Poland
Tel: 00800.7777.9999
Email: custserv.po@mpbio.com

Russia
Tel: +7 495 604.13.44
Email: custserv.rs@mpbio.com

Serbia
Tel: +381 11.242.1972
Email: custserv.se@mpbio.com

Singapore/ APAC
Tel: +65 6775.0008
Tel: +65 6394.7675
Email: custserv.ap@mpbio.com

South Korea
Tel: +82 2.425.5991
Email: custserv.kr@mpbio.com

Switzerland
Tel: 00800.7777.9999
Email: custserv.ch@mpbio.com

The Netherlands
Tel: 00800.7777.9999
Email: custserv.nl@mpbio.com

United Kingdom
Tel: 0800.282.474
Email: custserv.uk@mpbio.com

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