

INSTRUCTION MANUAL

YeaStar[™] RNA Kit

Catalog No. R1002

Highlights

- Recovery of purified RNA from a wide range of fungus species using Zymo-Spin[™] column technology.
- Eluted RNA is suitable for use in RT-PCR or other RNA-based procedures.
- Omits the use of glass beads and organic denaturants.

Contents

Product Contents	1	
Product Specifications	1	
Product Description	2	
Reagent Preparation	3	
Protocol	3	
Ordering Information	4	

For Research Use Only Ver. 1.0.3

Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please call 1-888-882-9682.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

YeaStar™ RNA Kit (Kit Size)	R1002 (40 Preps.)	Storage Temperature
YR Digestion Buffer	3.2 ml	Room Temp.
YR Lysis Buffer	6.4 ml	Room Temp.
RNA Wash Buffer ¹ (concentrate)	6 ml	Room Temp.
DNase/RNase-Free Water	6 ml	Room Temp.
Zymolyase ²	1000 U	-20°C
Zymolyase Storage Buffer	500 µl	-20°C
Zymo-Spin™ IIICG Columns	50	Room Temp.
Collection Tubes	50	Room Temp.
Instruction Manual	1	-

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

Specifications

- Sample Sources Fungi susceptible to yeast lytic enzyme lysis.
- Sample Size 1 to 1.5 ml liquid culture.
- Yield Typically, RNA is eluted into ≥35 μl RNase-free water. The RNA binding capacity of the supplied Zymo-Spin™ IIICG Columns is ~25 μg.
- RNA Purity High quality RNA suitable for all downstream manipulations.
- RNA Storage RNA is eluted with RNase-free water and can be stored at ≤-70 °C. The addition of RNase inhibitors is recommended for prolonged storage.
- Required Equipment Microcentrifuge.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

™ Trademarks of Zymo Research Corporation.

¹ Before starting, add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

² Before starting, add 200 μl **Zymolyase Storage Buffer** to the lyophilized enzyme for a final concentration of 5 U/μl.

Product Description

The **YeaStar**™ **RNA Kit** provides all the necessary reagents for RNA isolation from a broad spectrum of fungi including: *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus nivens var. aureus*, *Candida albicans*, *Pichia pastoris*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*. Generally, the kit can be used for the purification of high-quality, total RNA from any fungus that can be lysed by yeast lytic enzyme. The kit facilitates the purification of up to 25 µg total RNA from 1 to 1.5 ml of cultured cells using the Zymo-Spin™ column technology.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

Digest Yeast w/ Zymolyase Lytic Enzyme



Yeast Lysate



Ultra-pure RNA for ...

- √ Reverse Transcription
- ✓ Northern Blotting, etc.

Ensure the RNA isolation procedure is performed in an RNase-free environment.

Notes:

- ¹ The following protocol is designed for the purification of RNA from 1 to 1.5 ml cell culture (~1-5x10⁷ cells). RNA can be isolated from fresh or aged cells grown on solid or in liquid medium.
- ² The incubation time will depend on the cell number. Generally, if the cell pellet volume from *Step 1* is less than 25 μl, incubate for 40 minutes, if it is greater than 25 μl, incubate for 1 hour.
- ³ Elution efficiency is related to pH with the optimal being from 7.0 to 8.5. When water is used to elute the RNA, make sure the pH is above 6.0. Also, allow water to absorb into the column matrix before eluting the RNA. This will improve the RNA yield for RNAs >6 kb.

Reagent Preparation

- ✓ Before starting, add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml RNA Wash Buffer concentrate.
- Before starting, add 200 μl Zymolyase Storage Buffer to the lyophilized enzyme for a final concentration of 5 U/μl.

Protocol¹

All centrifugation steps should be performed at $10,000 - 16,000 \times g$ for 30 seconds unless specified. All steps should be performed at room temperature ($20-30^{\circ}$ C) unless specified.

- 1. Pellet 1-5 $\times 10^7$ cells (1-1.5 ml culture) by centrifugation at 500 $\times g$ for 2 minutes. Carefully remove all of the supernatant.
- Add 80 μl of the YR Digestion Buffer and 5 μl of the Zymolyase to the cell pellet and resuspend the pellet completely by pipetting. Incubate the suspension at 30-37°C for 40-60 minutes².
- 3. Add 160 µl of the YR Lysis Buffer and mix thoroughly by vortexing.
- 4. Add 1 volume ethanol (95-100%) to the sample and mix thoroughly.
- 5. Transfer the mixture to the **Zymo-Spin**^{$^{\text{M}}$} **IIICG Column** in a **Collection Tube** and centrifuge at $\geq 10,000 \text{ x } g$ for 1 minute.
- 6. Add 200 μ l **RNA Wash Buffer** to the column and centrifuge at \geq 10,000 x g for 1 minute. Discard the flow-through. Repeat the wash step.
- 7. Remove the Zymo-Spin IIICG Column carefully from the Collection Tube and transfer it into an RNase-free tube (not provided). Add 60 μl of DNase/RNase-Free Water directly to the column matrix³. Centrifuge at ≥10,000 x g for 30 seconds. The eluted RNA can be used immediately or stored at -70°C.

Ordering Information

Product Description	Kit Size	Catalog No.
YeaStar™ RNA Kit	40 Preps.	R1002

For Individual Sale	Amount	Catalog No.
YR Digestion Buffer	3.2 ml	R1001-1
YR Lysis Buffer	6.4 ml	R1001-2
RNA Wash Buffer (concentrate)	6 ml 12 ml 24 ml 48 ml	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48
DNase/RNase-Free Water	1 ml 6 ml 10 ml	W1001-1 W1001-6 W1001-10
Zymolyase (with storage buffer)	1,000 U 2,000 U	E1004 E1005
Zymo-Spin [™] IIICG Columns	50 250	C1006-50-G C1006-250-G
Collection Tubes	50 500 1,000	C1001-50 C1001-500 C1001-1000

RNA MADE SIMPLE

