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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.

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For Research Use Only

Ver. 1.0.3

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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.
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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.

[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.

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.

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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.

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

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.

Protocol[1]

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

1. Pellet 1-5 x10^7 cells (1-1.5 ml culture) by centrifugation at 500 x *g* for 2 minutes. Carefully remove all of the supernatant.

2. 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 minutes[2].

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™ IIICG Column** in a **Collection Tube** and centrifuge at ≥10,000 x *g* for 1 minute.

6. Add 200 µl **RNA Wash Buffer** to the column and centrifuge at ≥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 matrix[3]. Centrifuge at ≥10,000 x *g* for 30 seconds. The eluted RNA can be used immediately or stored at -70ºC.

Notes:

[1] The following protocol is designed for the purification of RNA from 1 to 1.5 ml cell culture (~1-5x10^7 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.

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

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