

INSTRUCTION MANUAL

YeaStar RNA Kit™

Catalog Nos. R1001 & R1002

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For Laboratory Use Only

I. General Information

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 10-25 µg of total RNA from 1-1.5 ml of cultured cells using Zymo Research's Fast-Spin column technology.

Highlights

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

Specifications

- **RNA Purity** High quality, purified RNA is recovered in the supplied RNase-free water.
- RNA Recovery Typically, RNA is eluted into 60 μl RNase-free water. The provided columns have an RNA binding capacity of 25 μg.
- Sample Sources Fungi susceptible to yeast lytic enzyme lysis.
- **Sample Size** 1 to 1.5 ml liquid culture.
- Stability of Product Reagents Integrity of kit components is guaranteed for up to one year from date of purchase.
- Quality Control Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

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

Note - ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. Some reagents included with this kit are chaotropic and are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

Product Contents

YeaStar RNA Kit™	R1001 Amount	R1002 Amount	Storage Temperature
YR Digestion Buffer	3.2 ml	3.2 ml	Room Temp.
YR Lysis Buffer	6.4 ml	6.4 ml	Room Temp.
RNA Wash Buffer*	6 ml	6 ml	Room Temp.
DNase/RNase-Free Water	4 ml	4 ml	Room Temp.
Zymolyase ^{™**}	1000 U	1000 U	-20°C
Zymolyase™ Storage Buffer	500 µl	500 µl	-20°C
Zymo-Spin III™ Columns (uncapped)	50 ct.	-	Room Temp.
Zymo-Spin IIIC [™] Columns (capped)	-	50 ct.	Room Temp.
Collection Tubes	50 ct.	50 ct.	Room Temp.
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* Ethanol must be added prior to use as indicated on RNA Wash Buffer label.
** Add 200 µl of the supplied Zymolyase[™] Storage Buffer to the lyophilized enzyme for a final concentration of 5 U/µl.

Ordering Information

Product Description	Catalog No.	Kit Size
YeaStar RNA Kit™ w/ uncapped columns	R1001	40 Preps.
YeaStar RNA Kit™ w/ capped columns	R1002	40 Preps.

For Individual Sale	Catalog No.	Amount
YR Digestion Buffer	R1001-1	3.2 ml
YR Lysis Buffer	R1001-2	6.4 ml
RNA Wash Buffer	R1003-3-2.4 R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	2.4 ml 6 ml 12 ml 24 ml 48 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10	1 ml 4 ml 6 ml 10 ml
Zymolyase™	E1004 E1005	1,000 U 2,000 U

For Individual Sale Cont.	Catalog No.	Amount
Zymo-Spin III™ Columns (uncapped)	C1005-50 C1005-250	50 ct. 250 ct.
Zymo-Spin IIIC™ Columns (capped)	C1006-50 C1006-250	50 ct. 250 ct.
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 ct. 500 ct. 1,000 ct.

II. Protocol

Reagent Preparation

- Before starting, add 24 ml 100% ethanol to the RNA Wash Buffer concentrate to obtain the final RNA Wash Buffer solution.
- Add 200 µl of the supplied **Zymolyase[™] Storage Buffer** to the lyophilized **Zymolyase[™]** enzyme for a final concentration of 5 U/µl.
- Make sure guidelines are followed to ensure the RNA isolation procedure is performed in an RNase-free environment.

Method

The following protocol is designed for the purification of RNA from 1 to 1.5 m cultured cells (approx. $1-5\times10^7$ cells). RNA can be isolated using this product from fresh or aged cells grown on/in solid/liquid medium, respectively.

- 1. Pellet 1-5 $\times 10^7$ cells (1-1.5 ml culture) by centrifugation at 500 x g for 2 minutes. Carefully remove all of the supernatant.
- To the cell pellet, add 80 µl of YR Digestion Buffer and 5 µl of Zymolyase[™]. Resuspend the pellet completely by repeated pipetting. Incubate the suspension at 30-37°C for 40-60 minutes.
- 3. Add 160 µl of YR Lysis Buffer and mix thoroughly by vortexing.
- 4. Centrifuge the mixture at 7,000 rpm in a microcentrifuge for 2 minutes.
- 5. Transfer the supernatant to a **Zymo-Spin Column** in a **Collection Tube** and centrifuge at ≥10,000 RPM for 1 minute.
- 6. Add 200 μ l **RNA Wash Buffer** to the column and centrifuge for 1 minute at \geq 10,000 rpm to wash. Discard the flow-through. Repeat wash step.
- Transfer the Zymo-Spin Column to a new RNase-free 1.5 ml centrifuge tube. Add 60 µl of DNase/RNase-Free Water directly to the column membrane. Centrifuge for 30 seconds at ≥10,000 RPM to elute the RNA.

For **Technical Assistance** contact those at **Zymo Research's Technical Department** at 1-888-882-9682 or E-mail to tech@zymoresearch.com.

Note: Alternatively 26 ml 95% ethanol can be added to the 6 ml wash buffer concentrate.

Note: 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 60 minutes.

Note: In most cases, a solid pellet will form following centrifugation. If the supernatant is overly viscous, vortex again, and centrifuge at $\geq 10,000$ rpm for 2 minutes. Continue onto the next step (5).

Note: 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

Popular RNA Purification & Isolation Products from Zymo

Product	Description	Kit Size	Cat No. (Column Format)
Pinpoint Slide RNA	Isolation of total RNA from targeted tissue areas	50 Preps	R1003
isolation system i m	sections. Perfect for RNA isolation from clinical		
	tissue samples.		
Pinpoint Slide RNA	Isolation of total RNA from targeted tissue areas	50 Preps	R1007
Isolation System II™	on a microscope slide of paraffin embedded tissue		
	sections. Perfect for RNA isolation from clinical		
	tissue samples.	EQ Drama	
MINI RNA	Isolation of trace amounts of RNA from 1x10 to	50 Preps	R1005 (Oncapped)
Isolation I Kit	uncapped column formats	50 Pieps	R1006 (Capped)
Mini RNA	Isolation of RNA from 10^2 to $5x10^6$ cells. Available	50 Preps	R1030 (Capped)
Isolation II Kit™	in both capped and uncapped column formats.	200 Preps	R1031 (Capped)
		50 Preps	B1032 (Uncapped)
		200 Preps	R1033 (Uncanned)
7R Whole Blood	Isolation of PNA from whole blood complex in 15	50 Prope	P1020
ZR Whole Blood Total RNΔ Kit™	minutes	100 Preps	R1020
	lastation of viral DNA fram call free body flyide on		R1021
ZR VIFALRNA KIT	Isolation of viral RNA from cell-free body fluids or sample mixtures containing cells at a	50 Preps	R1034
	concentration less than 10 ⁵ cells per ml	20011603	R1035
ZR-96 Viral RNA Kit™	High-output isolation of viral RNA from cell-free	2x96 Preps	R1040
	body fluids or sample mixtures containing cells at	4x96 Preps	R1041
	a concentration less than 10 ⁵ cells per ml.		
Urine RNA Kit™	Isolation of total RNA from urine sediment	20 Preps	R1038
	samples. The system employs a unique urine filter	50 Preps	R1039
	to collect cells via a simple syringe push-through		
RNA Clean-up Kit-5™	Clean and concentrate 5 ug RNA from any	50 Preps	R1015 (Capped)
	reaction in 2 minutes. 8 µl minimal elution	200 Preps	R1016 (Capped)
	volume. Available in capped and uncapped	50 Preps	R1023 (Uncapped)
	column format.	200 Preps	R1024 (Uncapped)
RNA Clean-un Kit-	Clean and concentrate up to 25 up RNA from any	50 Press	R1017 (Capped)
25 [™]	reaction in 2 minutes. 50 ul minimal elution	200 Preps	R1018 (Canned)
	volume. Available in capped and uncapped	50 Preps	P1025 (Upgapped)
	column format.	200 Preps	B1026 (Uncapped)
	Efficiently removes DNA from DNA proportions	EQ Drama	R1026 (Oncapped)
DNA Free RNA KIT	for PT PCP reactions in 15 minutes. Available in	200 Preps	R1013 (Capped)
	capped and uncapped column format	50 Preps	
		200 Preps	R1027 (Uncapped)
			R1028 (Uncapped)
Zymoclean Gel RNA	Isolation of RNA from agarose gels in 15 minutes.	50 Preps	R1011
Recovery Kit™	8 µl minimal elution volume for maximum		
	concentration of KNA.	40 Dropp	P1001 (Uppopped)
reastar KNA Kit'	suscentible to vesst lytic enzyme lysis	40 Pieps	
	susceptible to yeast tytic enzythe tysis.		K1002 (Capped)