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ZYMO RESEARCH

RNA
Purification
Made Simple

YeaStar™ RNA Kit

RNA from any tough-to-lyse sample

Highlights

- Quick, spin-column purification of total RNA (including small/microRNAs) from a wide range of fungi species, susceptible to yeast lytic enzyme lysis.
- Omits the use of mechanical homogenization and organic denaturants.
- RNA is ready for Next-Gen Sequencing, RT/qPCR and any downstream application, etc.

Catalog Numbers:
R1002



Scan with your smart-phone camera to
view the online protocol/video.



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Table of Contents

Product Contents	01
Specifications	02
Product Description	03
Protocol	04
(I) Buffer Preparation	04
(II) Total RNA Purification	05
Ordering Information	06
Complete Your Workflow	07
Notes	08
Guarantee	09

Product Contents

YeaStar™ RNA Kit	R1002 (40 prep)
YR Digestion Buffer	3.2 ml
YR Lysis Buffer	6.4 ml
RNA Wash Buffer ¹ (concentrate)	6 ml
DNase/RNase-Free Water	6 ml
Zymolyase ² (lyophilized)	1000 U
Zymolyase Storage Buffer	0.5 ml
Zymo-Spin™ IIICG Columns	50
Collection Tubes	50
Instruction Manual	1

Storage Temperature - Store all kit components (i.e., buffers, columns) at room temperature.

Before use:

1 Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer** concentrate.

2 Add 200 µl **Zymolyase Storage Buffer** to the lyophilized **Zymolyase** and mix by gentle inversion. Store frozen aliquots.

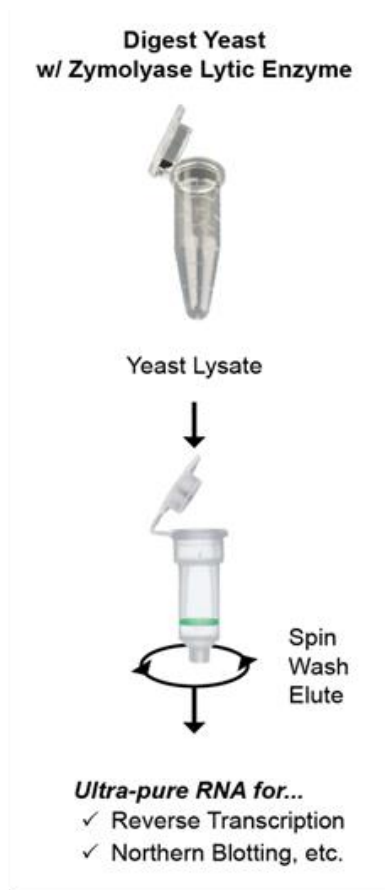
Specifications

- **Sample Sources** – Any fungi species ($1-5 \times 10^7$ pelleted cells from 1-1.5 ml culture), susceptible to yeast lytic enzyme lysis. RNA can also be isolated from aged cells grown on solid or in liquid medium. Specific sample types include, but are not limited to: *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus nivers var. aureus*, *Candida albicans*, *Pichia pastoris*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*.
- **Size** – Total RNA including small/microRNAs (≥ 17 nt).
- **Purity** – A_{260}/A_{280} & $A_{260}/A_{230} > 1.8$. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- **Binding Capacity** – **Zymo-Spin™ IICG Column** yield up to 100 μg RNA.
- **Elution Volume** – $\geq 35 \mu\text{l}$ **DNase/RNase-Free Water**.
- **Equipment Needed (user provided)** – Microcentrifuge, vortex.

Product Description

The **YeaStar™ RNA Kit** provides for rapid isolation of total RNA from a wide range of fungi species, susceptible to yeast lytic enzyme lysis including *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus nivers var. aureus*, *Candida albicans*, *Pichia pastoris*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*.

The **Zymo-Spin™ IICG Column** efficiently binds total RNA and is eluted in $\geq 35 \mu\text{l}$ water, suitable for subsequent procedures including RT-qPCR.



Protocol

The protocol consists of: (I) Buffer Preparation and (II) Total RNA Purification

(I) Buffer Preparation

- ✓ Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **RNA Wash Buffer**.
- ✓ Add 200 μl **Zymolyase Storage Buffer** to the lyophilized **Zymolyase** (final concentration 5 U/ μl) and mix by gentle inversion. Store frozen aliquots.

(II) Total RNA Purification

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.
- 1. Pellet $1-5 \times 10^7$ cells (1-1.5 ml culture) by centrifugation at 500 x g for 2 minutes. Then carefully remove the supernatant.
- 2. Add 80 μ l of **YR Digestion Buffer** and 5 μ l of **Zymolyase**¹ to the cell pellet and resuspend the pellet completely by pipetting up and down.
- 3. Incubate the suspension at 30-37°C for 40-60 minutes².
If the volume of the cell pellet is $\leq 25 \mu$ l, incubate for 40 minutes. For cell pellet volume $\geq 25 \mu$ l, incubate for 60 minutes.
- 4. Add 160 μ l of the **YR Lysis Buffer** and mix thoroughly by vortexing.
- 5. Add an equal volume ethanol (95-100%) (1:1) and mix well.
Example: Add 245 μ l ethanol to 245 μ l mixture (sample in **YR Lysis Buffer**).
- 6. Transfer the mixture to the **Zymo-Spin™ IIICG Column**³ in a **Collection Tube** and centrifuge. Discard the flow-through.
(Optional) At this step, DNase I treatment can be performed with the **DNase I Set** and **RNA Prep Buffer** (#E1010 and #R1060-2-25; available separately):
 - (D1) Wash column with 400 μ l **RNA Wash Buffer** and centrifuge. Discard flow-through.
 - (D2) In a nuclease-free tube, add 5 μ l **DNase I** (1 U/ μ l), 75 μ l **DNA Digestion Buffer** and mix. Add mixture directly into the column matrix.
 - (D3) Incubate the column at room temperature (20-30°C) for 15 minutes.
 - (D4) Add 400 μ l **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.
- 7. Add 200 μ l **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
- 8. Add 200 μ l **RNA Wash Buffer** to the column and centrifuge. Then carefully, transfer the column into a nuclease-free tube (not provided).
- 9. Add 60 μ l of **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use $\geq 35 \mu$ l elution.

The eluted RNA can be used immediately or stored frozen.

1 Prior to use, resuspend the lyophilized **Zymolyase** according to Buffer Preparation, page 4.

2. Incubation time will vary based on cell number. Optimization may be required.

3 To process samples > 700 μ l, columns may be reloaded.

Ordering Information

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

Individual Kit Components	Catalog No.	Amount
YR Digestion Buffer	R1001-1	3.2 ml
YR Lysis Buffer	R1001-2	6.4 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-24	6 ml 24 ml
DNase/RNase-Free Water	W1001-10 W1001-30	10 ml 30 ml
Zymolyase (lyophilized; with Storage Buffer)	E1004 E1005 E1006 (w/ RNase A)	1000 U 2000 U 1000 U
Zymo-Spin™ IIICG Columns	C1006-50-G	50
Collection Tubes	C1001-50	50

Complete Your Workflow

- ✓ For tough-to-lyse samples, use ZR BashingBead Lysis Tubes:

ZR BashingBead Lysis Tubes	
2.0 mm beads #S6003	Plant/animal tissue
0.1 + 0.5 mm beads #S6012	Microbes
0.1 + 2.0 mm beads #S6014	Microbes in tissue/insects

- ✓ For isolation of RNA from any sample:

Quick-RNA Fungal/Bacterial Kits	
Microprep #R2010	Includes ZR BashingBead Lysis Tubes for mechanical homogenization of tough-to-lyse microbial samples.
Miniprep #R2010	

- ✓ For clean-up (purification) and concentration of any RNA sample. (e.g., from the aqueous phase of TRIzol[®] extractions) or from any enzymatic reaction (e.g., DNase I treated RNA):

RNA Clean & Concentrator kits	
Microprep #R1013-R1014	DNase I Set included
MagBeads #R1082	Automatable (Tecan, Hamilton, Kingfisher, etc.)

- ✓ For NGS:

Zymo-Seq RiboFree Total RNA Library Prep kit	
#R3000	12 preps
#R3003	96 preps



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