



YeaStar[™] RNA Kit

RNA from any tough-to-lyse sample

Highlights

- spin-column purification of RNA Quick, total (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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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:

Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml RNA Wash Buffer concentrate.
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 x 10⁷ 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 nivens var. aureus, Candida albicans, Pichia pastoris, Saccharomyces cerevisiae, Schizosaccharomyces pombe.
- Size Total RNA including small/microRNAs (≥ 17 nt).
- Purity A₂₆₀/A₂₈₀ & A₂₆₀/A₂₃₀ > 1.8. RNA is ready for Next-Gen Sequencing, RT/qPCR, etc.
- Binding Capacity Zymo-Spin[™] IIICG Column yield up to 100 µg RNA.
- Elution Volume ≥ 35 µl 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 nivens var. aureus, Candida albicans, Pichia pastoris, Saccharomyces cerevisiae, Schizosaccharomyces pombe.

The **Zymo-Spin^{\mathcal{M}} IIICG Column** efficiently binds total RNA and is eluted in \geq 35 µl 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.
- Pellet 1-5 x 10⁷ cells (1-1.5 ml culture) by centrifugation at 500 x g for 2 minutes. Then carefully remove the supernatant.
- Add 80 μl of YR Digestion Buffer and 5 μl of Zymolyase¹ 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 µl 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 µl ethanol to 245 µl 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 µl RNA Wash Buffer and centrifuge. Discard flow-through.
- (D2) In a nuclease-free tube, add 5 μ l **DNase I** (1 U/µl), 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 μl RNA Prep Buffer to the column and centrifuge. Discard the flow-through.
- 7. Add 200 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.
- 8. Add 200 µl **RNA Wash Buffer** to the column and centrifuge. Then carefully, transfer the column into a nuclease-free tube (not provided).
- Add 60 µl of DNase/RNase-Free Water directly to the column matrix and centrifuge.

Alternatively, for highly concentrated RNA use \geq 35 µl 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
Miniprep #R2010	homogenization of tough-to-lyse microbial samples.

✓ 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

Notes



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