Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Yeast RNA Mini Kit

(Cat# K1418-50, -250; Ship at RT; Store at Multiple Temperature)

I. Introduction:

BioVision's Yeast RNA Mini Kit provides an easy and fast method for isolating total RNA from yeast within 30 min. Only trace genomic DNA exists in the purified RNA, which can be eliminated by DNAse I treatment when it is necessary.

II. Sample Type:

- For fast and efficient isolation of total RNA from yeast within 30 min.

III. Kit Contents:

Components	K1418-50 50 Preparations	K1418-250 250 Preparations	Part Number
Buffer LY*	28 mL	135 mL	K1418-XX-1
Buffer RB	28 mL	120 mL	K1418-XX-2
RNA Wash Buffer**	12 mL	50 mL	K1418-XX-3
DEPC-Treated ddH_2O	10 mL	30 mL	K1418-XX-4
Buffer SE	120 mL	500 mL	K1418-XX-5
RNA Columns with Tubes	50	250	K1418-XX-6
Lyticase	11,000 U	55,000 U	K1418-XX-7

*Add 1% volume of β-mercaptoethanol to Buffer LY before use and store at 4°C. Final 10 mM DTT can be used to replace β-mercaptoethanol. ***Add 48 mL (K1418-50) or 200 mL (K1418-250) 100% ethanol to RNA Wash Buffer before use.*

IV. User Supplied Reagents and Equipment:

- Microcentrifuge and 1.5 mL sterile tubes.
- Vacuum manifold if use vacuum protocol.
- 100% ethanol.
- β-mercaptoethanol.
- **Optional:** DNAse I

V. Shipment and Storage:

All the reagents are shipped at room temperature. DNAse I (optional), and Lyticase should be stored at -20°C. All other components can be stored at room temperature. All kit components are guaranteed for 24 months from the date of purchasing. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

Prepare all components and get all necessary materials ready by examining the protocol and become familiar with each step.

- Add 1% volume of β-mercaptoethanol to Buffer LY before use and store at 4°C. Final 10 mM DTT can be used to replace β-mercaptoethanol
- Add 48 mL (K1418-50) or 200 mL (K1418-250) 100% ethanol to RNA Wash Buffer before use.
- Prepare a Lyticase stock solution at 100 units/mL and aliquot into adequate portions. Store each aliquot at -20°C and thaw before use. Each sample will require 30 µL of this solution.
  (K1418-50), dissolve with 1.6 mL of SE Buffer
  (K1418-250), dissolve with 8 mL of SE Buffer for each bottle. ***Note: Perform all steps including centrifugation at room temperature.***

VII. Protocol for Extracting Total RNA From Yeast:

1. Grow cells in 3 mL selective media overnight at an appropriate temperature to an OD_{600} > 1. Pellet the cells in 1.5 mL microtubes for 5 min at 5000 rpm at 4°C. Use only freshly harvested cells for RNA isolation
2. Resuspend the pellet in 2 mL of Buffer SE/Lyticase mix and incubate at 30°C for 20 min. Inverting the tube every 10 min.
3. Pellet spheroblasts at 5000 rpm for 5 min at RT. Carefully aspirate and discard supernatant. Add **500 µL Buffer LY/** β-mercaptoethanol to the cell pellet. Vortex at max speed for 2 min. Spin at 12,000 rpm for 2 min and transfer the supernatant to a clean vial. *Optional: Add about 30 mg of glassbeads before vortexing.*
4. Add **½ volume 100% ethanol** into the lysate and pipet 5 times to mix the solution. Vortex briefly if any precipitations.
5. Transfer the solution to a RNA column and centrifuge at 12,000 rpm for 30 secs. Discard the collection tube with the flow-through and put the column back to the column.
6. Add **400 µL Buffer RB** to the column and centrifuge at 12,000 rpm for 1 min. Discard the flow-through.
7. *Optional: Add **50 µL DNAse I** (5U，RNase-free) Mixture onto the middle of the column and incubate at room temperature for 15 min. Add **500 µL Buffer RB** to the column and centrifuge at 14,000 rpm for 1 min. Discard the flow-through. Add **300 µL RNA Wash Buffer** to the column and centrifuge at 14,000 rpm for 1 min. Discard the flow-through.*
8. Add **500 µL RNA Wash Buffer** to the column and centrifuge at 12,000 rpm for 30 secs. Discard the flow-through.
9. Centrifuge the empty column at 13,000 rpm, with the lid open, for another 1 min. Discard the flow-through. It is critical to remove residual ethanol for optimal elution.
10. Place the column to a RNase-free 1.5 mL tube, add **50-100 µL DEPC-Treated ddH_2O** to the column and centrifuge at 13,000 rpm for 2 min. The RNA is in the flow-through liquid. Store the RNA solution at -20℃.

Note: It is highly recommended that RNA quality be determined before downstream applications. The quality of RNA can be assessed by denatured agarose gel electrophoresis with the ethidium bromide staining. Several sharp bands should appear on the gel including 28S and 18S ribosomal RNA bands as well as certain populations of mRNA and bands. If these bands smear towards lower molecular weight RNAs, then the RNA has undergone major degradation during preparation, handling or storage, RNA molecule less than 200 bases in length do not efficiently bind to the RNA column. A A_{260}/A_{280} ratio of 1.8-2.0 corresponds to 90-100% pure nucleic acid.

VIII. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Low A_{260}/A_{280} ratios	• Protein contamination • Guanidine Thiocyanate contamination	• Do a Phenol:Chloroform extraction. Loss of total RNA (up to 40%) should be expected. • Add 2.5 volumes of ethanol and 0.1M NaCl (final concentration) to precipitate RNA. Incubate for 30 min at –20°C. Centrifuge at 10,000g for 15 min at 4°C. Resuspend the RNA pellet in DEPC-treated water.
Low Yield	• RNA in sample degraded • The binding capacity of the membrane in the spin column was exceeded • Ethanol not added to buffer	• Freeze samples immediately in liquid nitrogen and store at -70°C after collect it. Prepare fresh culture. • Use of too much tissue sample exceeding the binding capacity of spin column will cause the decreasing of total RNA yield. • Add ethanol to the RNA Wash Buffer and DNase Stop Solution before purification.
Genomic DNA contamination	• Too much total RNA sample was used in RT-PCR • The sample may contain too much genomic DNA	• Reduce total RNA amount used in RT-PCR to 50-100 ng. • Reduce the amount of starting tissue in the preparation of the homogenate. Most tissues will not show a genomic DNA contamination problem at 30 mg or less per prep. • Reduce cell numbers to 1-2x10^5 or increase buffer volume and do multiple loadings to column.

FOR RESEARCH USE ONLY! Not to be used on humans.