Manual

MasterPure Yeast RNA Purification Kit

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MasterPure™ Yeast RNA Purification Kit is part of the Epicentre™ product line, known for its unique genomics kits, enzymes, and reagents which offer high quality and reliable performance.

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1. Introduction

The MasterPure™ Yeast RNA Purification Kit provides all of the reagents needed to purify RNA from cell types including: *Candida*, *Saccharomyces*, *Schizosaccharomyces* and filamentous fungi. The kit utilises a rapid desalting process[1] to remove contaminating macromolecules, avoiding toxic organic solvents, bead-beating and spheroplasting.

2. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
MasterPure Yeast RNA Purification Kit	1 kit*	MPY03100	Extraction Reagent for RNA	SS000401-D4	60 mL
			MPC Protein Precipitation Reagent	SS000399-D3	50 mL
			Proteinase K (50 µg/µL)	SS000099-D3	200 µL
			RNase-Free DNase I (1 U/µL)	E0013-1D1	500 µL
			2X T & C Lysis Solution	SS000402-D2	20 mL
			RiboGuard™ RNase Inhibitor (40 U/µL)	E0126-40D3	100 µL
			10X DNase Buffer (330 mM Tris-HCl [pH 7.8], 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT)	SS000272-D11	2 mL
			TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA)	SS000001-D2	7 mL

* The MasterPure Yeast RNA Purification Kit contains sufficient reagents to perform 100 purifications.

3. Product specifications

Storage: Store the Proteinase K, RiboGuard RNase Inhibitor and RNase-Free DNase I from the MasterPure Yeast RNA Purification Kit at -20 °C in a freezer without a defrost cycle. The rest of the kit may be stored at room temperature for ease of use.

Storage buffer: RNase-Free DNase I is supplied in a 50% glycerol solution containing 10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$ and 10 mM $CaCl_2$. Proteinase K is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 10 mM $CaCl_2$, 0.1% Triton® X-100 (Rohm & Haas) and 1 mM dithiothreitol (DTT).

Quality control: The MasterPure Yeast RNA Purification Kit is function-tested by purifying RNA from *Saccharomyces cerevisiae*. RNA quality and yield are assayed by agarose gel electrophoresis, spectrophotometry and use as a template for RT-PCR.

4. RNA purification protocol

A. RNA purification

1. Dilute 1 µL of 50 µg/µL Proteinase K into 300 µL of extraction reagent for RNA for each sample. A premix may be prepared for multiple samples.
2. Pellet cells by centrifugation and discard the supernatant. The optimal number of cells varies with the species, but 1-1.5 mL of a mid-log culture gives good results for many types of yeast.
3. Vortex mix 10 seconds to disperse the cell pellet.
4. Add 300 µL of Extraction Reagent for RNA containing the Proteinase K and mix thoroughly by vortexing.
5. Incubate at 70 °C for 10-15 minutes; vortex mix every 5 minutes. Shorter incubations of 5-10 minutes can yield nearly as much RNA.
6. Place the samples on ice for 3-5 minutes and add 175 µL of MPC Protein Precipitation Reagent to 300 µL of lysed sample (solution may become cloudy). Vortex vigorously for 10 seconds.
7. Pellet the debris by centrifugation for 10 minutes at 4 °C at ≥10,000 x g in a microcentrifuge.
8. Transfer the supernatant fluid to a clean microcentrifuge tube and discard the pellet.
9. Add 500 µL of isopropanol to the recovered supernatant fluid. Invert the tube 30-40 times.
10. Pellet the RNA by centrifugation at 4 °C for 10 minutes at ≥10,000 x g in a microcentrifuge.
11. Carefully pour off or aspirate the isopropanol without dislodging the RNA pellet. If removal of contaminating DNA is required, proceed with DNase I treatment in part B, otherwise, continue with this protocol. The MasterPure Yeast RNA Purification Kit extracts yeast DNA much less efficiently than RNA, and DNase I treatment may not be needed.
12. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipette.
13. Resuspend the RNA in 35 µL of TE Buffer.
14. Quantitate RNA by its absorbance at 260 nm, and obtain an A_{260}/A_{280} ratio. Yields have been in the range of 25-50 µg of RNA per mL of A_{600}=1.0 cultures of *S. pombe*, and A_{260}/A_{280} ratios have been greater than 2.0. Alternatively, determine RNA yields for small samples by fluorimetry.

B. Removal of contaminating DNA from RNA preparations

1. Remove all of the residual isopropanol with a pipette.
2. Prepare 200 µL of DNase I solution for each sample. Add 20 µL of 10X DNase Buffer to 175 µL of deionised water, then add 5 µL of RNase-Free DNase I.
3. Completely resuspend the nucleic acid pellet in 200 µL of DNase I solution.
4. Incubate at 37 °C for 10 minutes.
   Note: *Additional incubation (up to 30 min) may be necessary to remove all contaminating DNA.*
5. Add 200 µL of 2X T and C Lysis Solution; vortex mix for 5 seconds.

6. Add 200 µL of MPC Protein Precipitation Reagent (solution may become cloudy). Vortex mix 10 seconds; place on ice 3-5 minutes.
7. Pellet the debris by centrifugation at 4 °C for 10 minutes at ≥10,000 x g in a microcentrifuge.
8. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
9. Add 500 µL of isopropanol to the supernatant. Invert the tube 30-40 times.
10. Pellet the purified RNA by centrifugation at 4 °C for 10 minutes in a microcentrifuge at ≥10,000 x g.
11. Carefully pour off or aspirate the isopropanol without dislodging the RNA pellet.
12. Rinse twice with 70% ethanol, being careful to not dislodge the pellet. Centrifuge briefly if the pellet is dislodged. Remove all of the residual ethanol with a pipette.
13. Resuspend the RNA in 35 µL of TE Buffer.
14. Add 1 µL of RiboGuard™ RNase Inhibitor.

5. References

1. Miller, S.A. et al., (1988) *Nucl. Acids Res. 16*, 1215.

6. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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