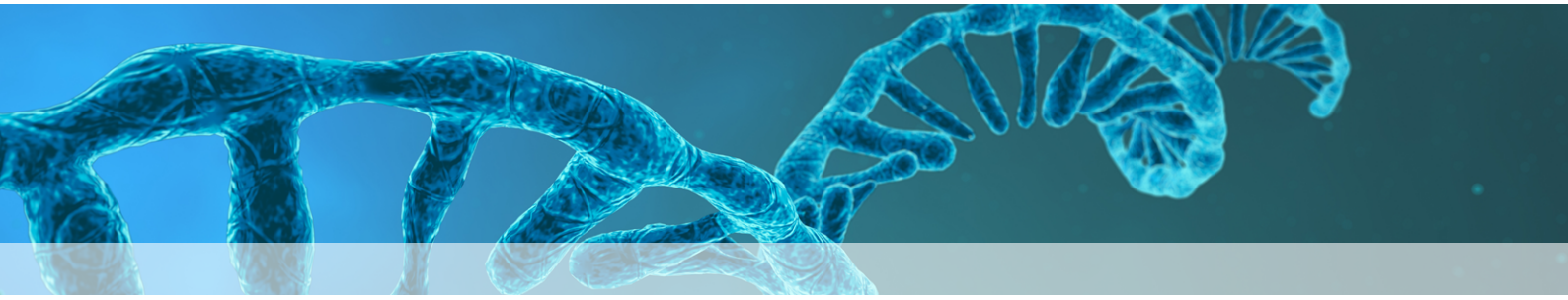
MACHEREY-NAGEL

RNA Isolation from Bacteria and Yeast

Recommendations for efficient sample disruption to facilitate RNA extraction from bacteria and yeast using MN Bead Tubes on Retsch mill or with MN Bead Tube holder on a Vortex Genie.



Introduction

RNA isolation is highly complicated by the presence of ubiquitous RNases that degrade RNA samples. Furthermore, the physical and biochemical structure of sample materials makes RNA purification more difficult e.g. in bacteria and yeast. Therefore, bead beating is an effective process used to disrupt different biological samples. Using MN Bead Tubes lead to high yields of non-degraded RNA. The parameters in Table 1 are recommended for RNA isolation from bacteria and yeast using the NucleoSpin® RNA kit.

Sample Material		Retsch mill		Bead Tube Holder	
	Bead Tubes	Speed	Time	Speed	Time
Gram-negative Bacteria e.g. *E. coli*	MN Bead Tubes Type B	30 Hz	1 min	Full speed	3 min
Gram-positive Bacteria e.g. *B. subtilis*	MN Bead Tubes Type B	30 Hz	1 min	Full speed	3 min
Yeast e.g. *K. pastoris*	MN Bead Tubes Type B	30 Hz	10 sec	Full speed	3 min

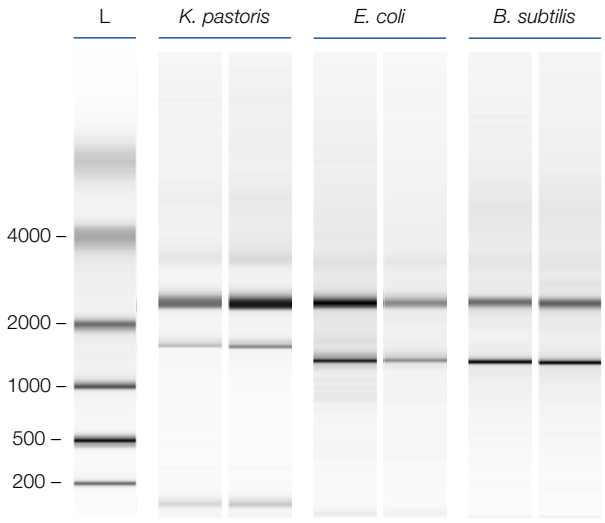
Table 1 Recommended parameters for RNA

The specified conditions resulted in the best yield with best RNA integrity. RNA Isolation was performed using a 25–30 mg wet-weight cell pellet and described as stated below.

Procedure		
1	Cell Harvest	Pellet cells (appx. 30 mg wet weight) by centrifugation and discard supernatant.
2	Resuspension	Add 350 µL Buffer RA1 to the cell pellet and resuspend by vortexing vigorously. Reducing agent is not required.
3	Homogenization and Lysis	Transfer the resuspended cells into a MN Bead Tube Type B and close the tube. Perform bead beating using a Retsch mill or MN Bead Tube Holder applying stated settings from table 1.
4	Sedimentation of Beads	Centrifuge the MN Bead Tube for 1 min at 11,000 x g to sediment the beads. Recover the supernatant (lysate). Proceed with step 3 of the NucleoSpin® RNA standard protocol (user manual, section 5.1).

Application data

A



B

Bead Beating in Bead Tube Holder	*K. pastoris*	*E. coli*	*B. subtilis*
Yield [µg] (RiboGreen)	16.1	8.0	11.9
OD 260/280	2.2	2.3	2.2
OD 260/230	2.5	2.4	2.4
RIN	8.9	8.8	8.8
Yield DNA [µg] (PicoGreen)	0.2	0.1	0.1

RNA yield and quality using Bead Tube Holder

RNA was purified from about 25–30 mg gram-negative (*E. coli*), gram-positive bacteria (*B. subtilis*) and yeast (*K. pastoris*) using the NucleoSpin® RNA kit (REF 740955) in combination with MN Bead Tubes Type B on the MN Bead Tube holder.

Eluates were analyzed by Bioanalyzer resulting in clearly defined ribosomal bands (A). The RNA was of high quality based on OD measurement as well as RIN value. In addition, the contamination with DNA was negligible (B).

C

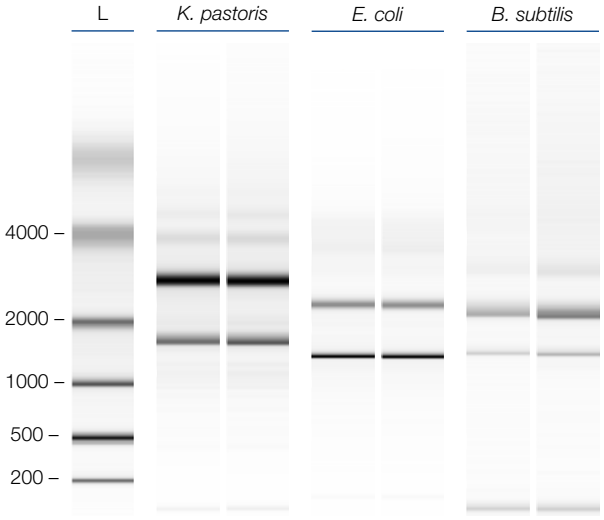
Bead Beating in Retsch mill	*K. pastoris*	*E. coli*	*B. subtilis*
Yield [µg] (RiboGreen)	8.3	41.0	16.0
OD 260/280	1.9	2.1	2.1
OD 260/230	1.9	2.4	2.3
RIN	7.9	9.3	9.0
Yield DNA [µg] (PicoGreen)	0.2	1.0	0.7

RNA yield and quality obtained using a Retsch mill for disruption

The RNA was isolated in the analogous manner to the procedure in the Bead Tube Holder, except for the mechanical disruption in the Retsch mill.

Also in conjunction with the Retsch mill, the analysis of purified RNA leads to clearly definded ribosamal bands when analyzed by Bioanalyzer (D) and provides good values for the integrity and purity of the RNA (C).

D



Ordering information

Product	Specifications	Pack of	REF
NucleoSpin® RNA	Mini spin kit for isolation of RNA of highest integrity	10 preps 50 preps 250 preps	740955.10 740955.50 740955.250
MN Bead Tubes Type B	2 mL tubes with 40–400 µm glass beads; for homogenization of Gram-positive and Gram-negative bacteria and yeast	50 pieces	740812.50
MN Bead Tube Holder	Rubber-foam adapter for processing MN Bead Tubes with Vortex-Genie 2	1 piece	740469