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Application Note

Pall Nucleic Acid Binding (NAB) Nanosep® Centrifugal Device Ensures High Quantity and Quality of the Isolated Bacterial Ribonucleic Acid (RNA)

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Introduction

Molecular exploration in bacteria has undergone a substantial change over the past decade. Emerging evidences indicate that the complex signal coordination around riboswitches, RNA thermometers and small bacterial RNAs is regulated by RNAs. Moreover, the growing interest in human host-pathogens interactions has fast-tracked the transition of metagenomic next generation sequencing (mNGS) from a research tool into a valuable clinical tool. In all these cases the journey begins with high quality RNA.

The quantity and quality of the input RNA determines the accuracy and reproducibility of an experiment. Depending on the demand for the quality of the input RNA, either a spectrophotometric, fluorometric, or capillary electrophoresis is performed to assess quality control on input nucleic acids. RNA is extremely susceptible to degradation because of the omnipresent RNase in the environment and precautions must be taken for successful extraction of high-quality RNA.

In this scientific brief we evaluate the performance of the the Pall Nucleic acid binding (NAB) Nanosep centrifugal device, which contains a dual layer silica-based quartz glass fiber media, for nucleic acid isolation and compare it with two other commercially available spin devices. All centrifugal devices chosen were silica-based spin devices and were utilized for lysis-bind-wash-elute method of nucleic acid purification. One of the devices is not part of a kit and does not have its own buffers (herein will be referred to as ""AA"), whereas the other centrifugal device is from a total RNA isolation kit (herein will be referred to as "BB") having its own buffers and manufacturer's recommended protocol. Total RNA extraction buffers (herein will be referred to as Brand "X") from a leading kit manufacturer were used to test performance of "AA" spin devices and NAB Nanosep spin device. The results indicate that the RNA isolated from *E. coli* TOP10 strain using Pall NAB Nanosep centrifugal device is either superior or equivalent to the commercially available brands AA and BB.

Materials and Methods

1. Sampling

RNA was extracted from log phase cultures of ***E. coli*** TOP10 cells. For each spin device, 2 mL of culture was initially used per centrifugal device for RNA extraction/purification, at OD600nm. This corresponds to 5 - 8 x 10^9 cells.

2. RNA Extraction procedure from E. coli TOP10 cells. Pall NAB Nanosep and Universal Spin Devices

Pall NAB Nanosep spin device centrifugal device and the commercially available centrifugal device named "AA" are known in the industry as a universal device suitable for purification of RNA, genomic DNA and plasmid DNA and are available without extraction buffers. Total RNA was isolated using a commercially available kit for total RNA isolation (Buffer X). The chosen kits have shown to be compatible and effective previously in an independent study (data not shown). Total RNA was eluted in a final volume of 50 µL.

3. Pall NAB Nanosep centrifugal device and BB centrifugal device with buffers kit.

Procedures for RNA extraction in Pall NAB Nanosep centrifugal device and the commercially available centrifugal device named "BB" were followed per manufacturer guidelines with the buffers supplied with column BB incorporating the following modifications. Each sample was incubated for 30 minutes at 22-25 °C with 200 µL of lysozyme (15 mg/mL stock concentration) and 10 µL of proteinase K (600 mAU/mL) to improve cell lysis and RNA extraction. The elution time was doubled from 1 to 2 mins to ensure a higher RNA yield from both devices.

4. RNA Quantification and Quality control

RNA was quantified after dilution in the Tris/HCl buffer (10 mM, ≈ pH 7) and the absorbances at 260 nm and 280 nm were recorded to calculate the purity ratio at 260/280 nm.

Results and Discussion

Pall NAB Nanosep and Universal Spin Devices

Pall NAB Nanosep and Brand "AA" spin devices were evaluated for extraction capacity using a commercially available kit containing buffers for total RNA isolation. The same pool of lysed *E. coli* TOP10 strain was used as input for both devices. As indicated in Table 1. NAB Nanosep spin devices showed higher extraction capacity with acceptable repeatability and purity. Results obtained from spectrophotometric analysis of the individual tubes tested are shown in Figure 1.

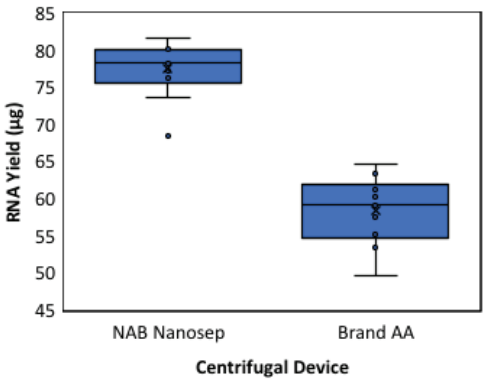
Table 1

RNA yield obtained after extraction from *E. coli* TOP10 strain using NAB Nanosep and Brand "AA" spin devices with commercially available buffer X for total RNA extraction. Data represents mean ± SD of 10 devices of Pall and 10 devices of Brand "AA".

	RNA NAB Nanosep	Brand "AA"
Quantity (µg)	77.6 ± 3.9	58.4 ± 4.6
*$A_{260/280}$	2.1 ± 0.1	2.1 ± 0.1
Culture volume	2 mL	
Cell number/sample	8×10^9	
Avg OD at 600 nm	0.8	

Figure 1

RNA quantity of samples isolated from 8×10^9 *E. coli* TOP10 cells were determined by spectrophotometric method. RNA was isolated with Pall NAB Nanosep and Brand "AA" spin filters using commercially available reagents (buffer X) for total RNA isolation. Data represents Pall NAB (n=10) and Brand "AA" (n=10) devices



Pall NAB Nanosep centrifugal device and centrifugal device with buffers kit.

Pall NAB Nanosep and Brand "BB" spin devices were evaluated for extraction capacity using the buffers supplied by Brand "BB". Results shown in Table 2 indicate that the extraction capability of both NAB Nanosep and Brand "BB" spin devices using the supplied buffer is similar. Both repeatability and purity of the sample extracted were within the acceptable range. Results obtained from spectrophotometric analysis of the individual tubes tested are shown in Figure 2.

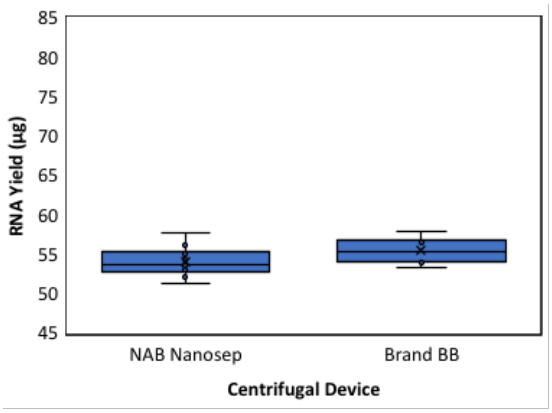
Table 2

RNA yield obtained after extraction from *E. coli* TOP10 strain using NAB Nanosep and Brand "BB" spin devices with total RNA isolation buffers provided with brand "BB". Data represents mean ± SD of 10 devices of Pall and 10 devices of Brand "BB".

	RNA NAB Nanosep	Brand "BB"
Quantity (µg)	54.1 ±1.9	55.5±1.6
*$A_{260/280}$	2.1±0.1	2.1±0.1
Culture volume	2 mL	
Cell number/sample	5.7×10^9	
Avg OD at 600 nm	0.6	

Figure 2

RNA quantity of samples isolated from 5.7×10^9 *E. coli* TOP10 cells were determined by spectrophotometer. RNA was isolated with Pall NAB Nanosep and Brand "BB" spin filters using a commercially available reagent for total RNA isolation. Data represents Pall (n=10) and Brand "BB" (n=10) devices



RNA extraction with NAB Nanosep, Brand "AA" and Brand "BB" with the same set of buffers.

RNA extraction was performed with NAB Nanosep spin devices and spin devices from Brand "AA" and "BB" using either Buffer "X" or Buffer "BB". This was done to eliminate any favorability that may affect the data in the choice of buffers and in terms of starting cell concentration. The extraction in this set was done using the same pool of lysed *E. coli* TOP10 and the same procedure. Results summarized in Table 3 indicate that two different buffers were tested, and the results appear to be consistent, suggesting that the Pall product was not unfairly favored by choice of buffer.

Table 3

RNA extraction with Pall NAB Nanosep, Brand "AA" and Brand "BB" spin devices with 2 mL of *E. coli* culture per sample at similar OD_{600} per set of buffers. Each data point represents the mean ± SD of 5 devices of each brand.

Buffer		"X"	"BB"
NAB Nanosep	Quantity (µg)	49.4 ± 2.0	41.6 ± 1.1
	*$A_{260/280}$	2.1 ± 0.1	2.1 ± 0.1
	Quantity (µg) in Table 1 & 2	77.6 ± 2.0	54.1 ± 1.9
Brand AA	Quantity (µg)	40.4 ± 0.9	38.4 ± 1.8
	*$A_{260/280}$	2.1 ± 0.1	2.1 ± 0.1
	Quantity (µg) in Table 1	58.4 ± 4.6	N/A
Brand BB	Quantity (µg)	46.5 ± 1.2	42.7 ± 1.9
	*$A_{260/280}$	2.1 ±0.1	2.1 ± 0.1
	Quantity (µg) in table 2	N/A	55.4 ± 1.6

* $A_{260/280}$ for pure RNA is expected at 1.9/2.0-2.2.

Summary

The aim of this study was to assess the efficiency of NAB Nanosep centrifugal devices to purify RNA from bacteria. It was evident from the experimental outcomes that it demonstrated robust performance using total RNA isolation protocols obtained from commercially available kits or buffer manufacturers. The RNA isolated with Pall NAB Nanosep spin devices are pure as indicated by 260/280 ratio and therefore is suitable for use in downstream applications.

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