PALL LABORATORY

Technical Bulletin

Nucleases - Are They a Problem for NAB Nanosep® Centrifugal Devices?

Summary

Sample tubes and collection tubes of Pall Nucleic Acid Binding (NAB) Nanosep centrifugal devices were assessed for the presence of DNase and RNase. Also evaluated for comparison were sample tubes of centrifugal devices obtained from commercially available kits from one manufacturer (COM1) for isolation of RNA, genomic DNA (gDNA), and plasmid DNA (pDNA), respectively (COM1-RNA, COM1-gDNA, and COM1-pDNA) as well as sample tubes of a centrifugal device commercially available without reagent (COM2).

No DNase or RNase contamination was detectable in Pall devices.

No DNase contamination was observed in any of the other devices. The chances of encountering an RNase contaminated device in general appears very small at worst as only a single COM2 sample tube out of the total of 70 devices tested (Pall, COM1, and COM2 devices) had a reading that just exceeded the strictest threshold for RNase contamination.

Introduction

Nucleases – enzymes that cleave the phosphodiester bonds of nucleic acids – can be a researcher's friend or foe.

Nucleases are essential and play an important role in nucleic acid metabolism involved in DNA damage repair, messenger RNA turnover. Many of the enzymes have become indispensable tools for research. Bacterial restriction endonucleases, which form a defense mechanism against invading viruses by digesting specific DNA sequences, have enabled researchers to cut and paste genetic sequences and now are commonly used for cloning of sequences into plasmid DNA vectors. DNase I, an enzyme that nonspecifically cleaves DNA to release 5'-phosphorylated di-, tri-, and tetra- nucleotides, is used in genomic research to map areas of open, accessible chromatin[1], and has found medical use in the treatment of cystic fibrosis[2].

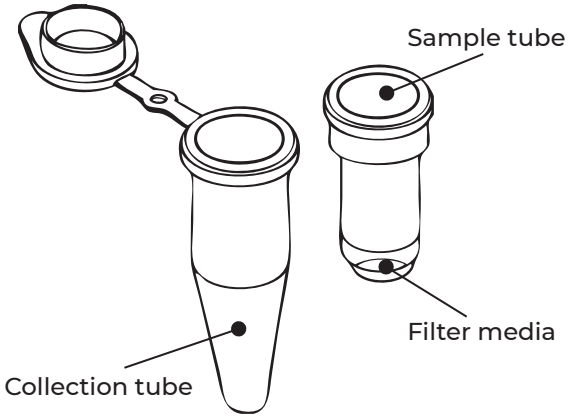
RNase can be added to plasmid DNA preparations to remove unwanted RNA molecules that copurified. However, small amounts of nucleases such as DNase and RNase can negatively impact samples if they are inadvertently introduced into precious DNA or RNA samples where they can reduce yield or worse destroy the sample altogether. RNA is more sensitive to degradation and small amounts of ribonucleases can copurify with isolated RNA and compromise downstream applications. Due to its ubiquitous presence in our environment, RNase contamination can also be introduced via tips, tubes, and other reagents used in procedures. Researchers working with RNA containing samples generally go to great lengths to remove any possible RNase contamination from their workplace by wiping benchtops with cleaning solutions such as RNaseZap◆ and by treating glassware and solutions with inactivating agents such as diethyl pyrocarbonate (DEPC)[3]. Furthermore, RNase inhibitors, (recombinant) proteins which inhibit RNase through tight noncovalent interaction, can be added to samples to protect them during experiments or storage.

Pall NAB Nanosep centrifugal devices are used for the isolation of plasmid DNA, genomic DNA, and RNA, but are not labeled "RNase-free" or "DNase-free". Here we examine whether the sample tubes and collection tubes of the NAB Nanosep centrifugal devices contain detectable amounts of DNase or RNase and for comparison also examine sample tubes of centrifugal devices obtained from commercially available kits from one manufacturer (COM1) for isolation of RNA, genomic DNA (gDNA), and plasmid DNA (pDNA), respectively (COM1-RNA, COM1-gDNA, and COM1-pDNA) as well as sample tubes of a centrifugal device commercially available without reagent (COM2).

No DNase was detectable in Pall devices or any of the other investigated devices. No RNase was detectable in Pall devices. Only a single COM2 sample tube out of the total of 70 devices tested (Pall, COM1, and COM2 devices) had a reading that just exceeded the strictest threshold for RNase contamination. Therefore, the chances of encountering an RNase contaminated centrifugal device in general appear very small at worst.

Figure 1

Pall NAB Nanosep centrifugal device sample tube and collection tube



Materials and Methods

Sample tubes from three batches (FE9081, FG0946, and FG1769) of NAB Nanosep centrifugal devices and NAB Nanosep receiver tubes were evaluated for the presence of DNase and RNase. Also evaluated for comparison were sample tubes from centrifugal devices obtained from commercially available kits for isolation of plasmid DNA, genomic DNA, and RNA (referred to from hereon as COM1-pDNA, COM1-gDNA, and COM1-RNA) as well as sample tubes from centrifugal devices commercially available from another manufacturer without reagents (COM2). The assays included Minus-nuclease control samples to set the assay base line and threshold criteria for contamination. In addition, Plus-nuclease samples were included to verify assay performance and range.

Prior to carrying out the experiments, equipment and materials were cleaned with RNaseZap to remove any traces of nucleases. For the nuclease assays, sample tubes of the centrifugal devices were filled with nuclease-free water and incubated at room temperature for 30-45 min. In line with the device sample capacity, for the Pall devices (sample tubes and receiver tubes) 500 µL aliquots of nuclease-free water were used, whereas 700 µL aliquots were used with the COM1 and COM2 devices. Table 1 presents the number of devices that were tested and the number of replicates of the Minus-nuclease and Plus-nuclease control samples.

Table 1

Replicate numbers for the DNase and RNase assays

	DNase Assay	RNase Assy
Minus-nuclease control	10	5
Plus-nuclease control	2	2
Pall NAB Nanosep collection tube	12	6
Pall NAB Nanosep sample tube lot FE9081	10	10
Pall NAB Nanosep sample tube lot FG0946	10	10
Pall NAB Nanosep sample tube lot FG1769	10	10
COM1-RNA sample tube	10	10
COM1-gDNA sample tube	10	10
COM1-pDNA sample tube	10	10
COM2 sample tube	10	10

DNase assays

RNase assays were carried out with the DNaseAlert◆ QC System (Fisher Scientific) according to manufacturer's instructions. The samples and reagents were added to wells of a Nunc◆ F96 MicroWell◆ black polystyrene plate (Fisher Scientific) as shown in Table 2. After this, the plate was covered with sealing film to minimize evaporation during incubated at 37 °C for 60 min.

Table 2

Sample and controls preparation for DNase assay

Reagent	Minus-DNase Controls	Plus-DNase Controls	Experimental Samples
DNaseAlert substrate	10 µL	10 µL	10 µL
10X NucleaseAlert buffer	10 µL	10 µL	10 µL
1X NucleaseAlert buffer		4 µL	
Nuclease-free water	80 µL	75 µL	
Sample			80 µL
DNase I (2U/µL)		1 µL	
Total	100 µL	100 µL	100 µL

Upon completion of the incubation, the well contents were collected at the bottom of the plate by centrifugation at 500 x g for 2 min. Fluorescence measurements took place in an Infinite◆ M200 multimode plate reader (Tecan) with an excitation wavelength of 530 nm and an emission wavelength of 580 nm.

RNase assays

RNase assays were carried out with the RNaseAlert◆ Lab Test Kit v2 (Fisher Scientific) according to manufacturer's instructions. The RNaseAlert substrate in this kit is provided lyophilized in 0.5 mL tubes to which reagents were added as shown in table 3.

Table 3

Sample and controls preparation for RNase assay

Reagent	Minus-RNase Controls	Plus-RNase Controls	Experimental Samples
RNaseAlert substrate lyophilized in 0.5 mL tube	Lyophilized	Lyophilized	Lyophilized
10X NucleaseAlert buffer	5 µL	5 µL	5 µL
Nuclease-free water	45 µL	40 µL	
Sample			45 µL
RNase A		5 µL	
Total	50 µL	50 µL	50 µL

Upon addition and mixing of the contents by vortexing, the tubes were incubated at 37 °C for 45-60 min. Upon completion of the incubation, just before fluorescence measurement, the volume in the tubes was brought up to 100 µL with nuclease-free water. The content of the tubes was brought down by centrifugation at 500 x g for 2 min. The samples were then transferred to wells of a Nunc F96 MicroWell black polystyrene plate (Fisher Scientific) and fluorescence measurements took place in an Infinite M200 multimode plate reader (Tecan) with an excitation wavelength of 490 nm and an emission wavelength of 520 nm.

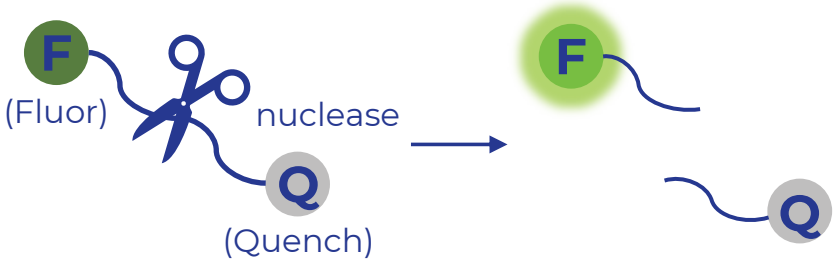
Results and Discussion

Sample tubes from three batches (FE9081, FG0946, and FG1769) of NAB Nanosep centrifugal devices and NAB Nanosep receiver tubes were evaluated for the presence of DNase and RNase. For comparison, sample tubes from centrifugal devices obtained from commercially available kits for isolation of plasmid DNA, genomic DNA, and RNA (COM1-pDNA, COM1-gDNA, and COM1-RNA) as well as sample tubes from centrifugal devices from another manufacturer that are commercially available without reagents (COM2).

As shown in Figure 2, the DNase and RNase assays use as substrate an oligonucleotide that is dual-labeled with a fluorescent reporter and a quencher. Cleavage of the oligonucleotide allows the reporter a fluorescent signal to fluoresce, and therefore the intensity of fluorescence is a measure of DNase or RNase contamination. The threshold for contamination is a 2 to 2.5-fold increase in fluorescence compared to the fluorescence obtained with Minus-nuclease control samples. The assay is considered to perform as expected when the Plus-nuclease samples fluoresce ≥20-fold above the Minus-nuclease control background.

Figure 2

Nuclease Assay Principle.



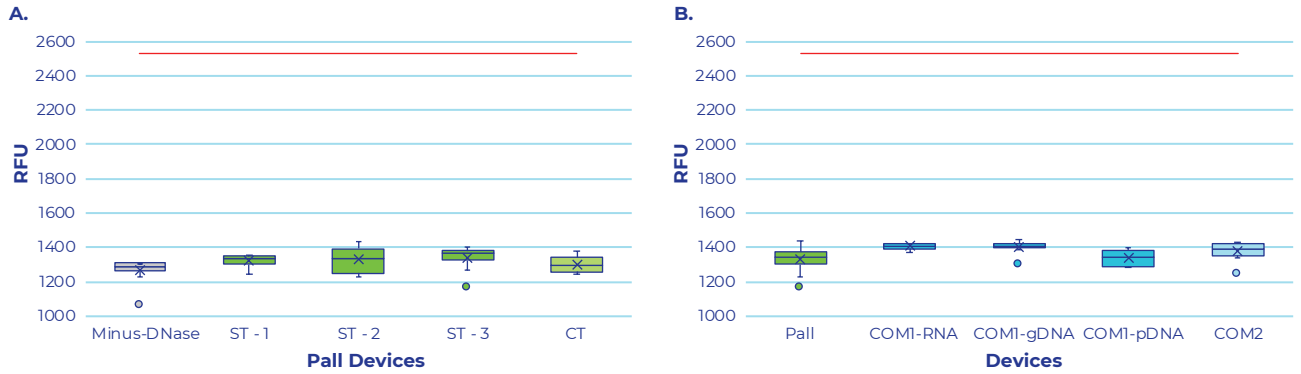
The substrate of the assays consists of a reporter (F)-quencher (Q) dual-labeled oligonucleotide. Cleavage of the oligonucleotide by the nuclease unquenches the reporter with a resulting increase in fluorescence. For the DNase assay, the oligonucleotide is a DNA oligo whereas it is an RNA oligo for the RNase assay.

DNase assays

The results of the DNase assays are shown in Figure 3 where panel A shows the results obtained with the Minus DNase control (n=10) compared against the sample tubes from three individual lots of NAB Nanosep devices (n=10 each) and NAB Nanosep collection tubes (n=12). In panel B, the aggregate of the NAB sample tubes (n=30) is compared against the sample tubes from COM1 and COM2 devices (n=10 each. With an approximately 35-fold difference between the average RFU value of the Minus-DNase control samples (1,266 ± 74) and the Plus-DNase control samples (44,288 ± 1,260), the assay was deemed to perform well (acceptance criterium is a difference ≥20-fold). All groups showed a relatively tight distribution in RFU values and all experimental groups, with exception of the Pall Nanosep sample tubes, showed a small, but statistically significant ($P < 0.05$ in Student's t-test assuming unequal variance) increase in RFU values. However, for all experimental sample groups, the values obtained were well below the threshold for contamination (red line, representing an RFU of 2,532), which was defined as twice the assay average RFU value of the negative control samples. Therefore, it can be concluded that no detectable DNase contamination was observed in Pall devices or any of the other tested samples.

Figure 3

Box plots of DNase assay results.



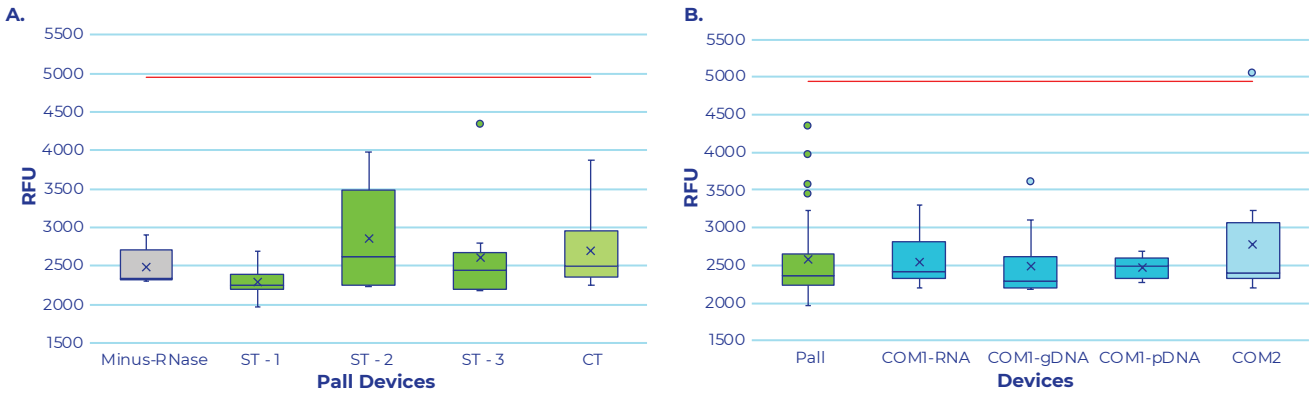
The box indicates samples ranging from first to third quartile, with the horizontal line representing the sample median and x the sample average. The whiskers represent samples falling within 1.5 times the interquartile range and the circles indicate outlier samples falling outside this range. Panel A shows results for Pall NAB Nanosep collection tubes (CT; n=12) and three lots of sample tubes (ST-1, ST-2, and ST-3; n=10 for each lot). A Minus-DNase negative control (n=10) is shown for comparison and is used to determine the DNase contamination threshold (red line) in both panels. Panel B shows the results for sample tubes of the combined three lots of Pall devices (Pall; n=30) shown in panel A and the COM1 and COM2 devices (n=10 each).

RNase assays

The results of the RNase assays are shown in Figure 4 where panel A shows the results obtained with the Minus-RNase control (n=5) compared against the sample tubes from three individual lots of NAB Nanosep devices (n=10 each) and NAB Nanosep collection tubes (n=6). In panel B, the aggregate of the NAB sample tubes (n=30) is compared against the sample tubes from COM1 and COM2 devices (n=10 each. The assay performed well with a nearly 27-fold difference between the average RFU value of the Minus-RNase control samples (2,476 ± 251) and the Plus-RNase control sample (65,380), the assay was deemed to perform well (acceptance criterium is a difference ≥20-fold). Out of the 70 sample tubes tested, only a single sample tube of one the COM2 devices, had an RFU value of 5,056, just above the threshold for contamination (red line, representing an RFU of 4,952), which was defined as twice the assay average RFU value of the negative control samples. This was measured by the strictest of standards as the test allows this parameter to be set as high as 2.5-fold above baseline. None the experimental groups were statistically distinguishable from the negative control samples ($P > 0.05$ in Student's t-test assuming unequal variance). Therefore, it can be concluded that no detectable RNase contamination was observed in Pall devices and that the chances of encountering a contaminated device in general are, at worst, very small as only a single COM2 sample tube had a fluorescence just barely above the strictest threshold for RNase contamination.

Figure 4

Box plots of RNase assay results.



The box indicates samples ranging from first to third quartile, with the horizontal line representing the sample median and x the sample average. The whiskers represent samples falling within 1.5 times the interquartile range and the circles indicate outlier samples falling outside this range. Panel A shows results for Pall NAB Nanosep collection tubes (CT; n=6) and three lots of sample tubes (ST-1, ST-2, and ST-3; n=10 for each lot). A Minus-RNase negative control (n=5) is shown for comparison and is used to determine the DNase contamination threshold (red line) in both panels. Panel B shows the results for sample tubes of the combined three lots of Pall devices (Pall; n=30) shown in panel A and the COM1 and COM2 devices (n=10 each).

Conclusions

Sample tubes from three batches of NAB Nanosep centrifugal devices and NAB Nanosep receiver tubes were evaluated for the presence of DNase and RNase. For comparison, sample tubes from centrifugal devices obtained from commercially available kits for isolation of plasmid DNA, genomic DNA, and RNA as well as sample tubes from centrifugal devices from another manufacturer (COM2) that are commercially available without reagents.

No detectable DNase contamination was observed in Pall devices or any of the other tested samples.

No detectable RNase contamination was observed in samples of Pall devices and the chances of encountering a contaminated device in general appears very small at worst as only a single COM2 sample tube had a fluorescence value just barely above the strictest threshold for RNase contamination.

References

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