Application Note

Isolation of Genomic DNA and RNA with Pall AcroPrep™ Advance 96-well Nucleic Acid Binding Filter Plates Using Commercially Available Reagents

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1. Introduction

Pall AcroPrep Advance 96-well Nucleic Acid Binding Filter plates (Pall NAB plate) offer researchers the flexibility to use a single plate for purification of plasmid DNA from bacteria[1,2], and genomic DNA (gDNA) or total RNA from cell culture samples[3,4]. In the referenced application notes, protocols were used employing in-house prepared reagents for plasmid DNA and RNA purification as well as protocols employing commercial reagents for plasmid DNA, genomic DNA, and RNA purification.

In this application note, we have sought to demonstrate the versatility of use of the Pall NAB filter plate for preparation of genomic DNA and RNA from cultured mammalian cells using commercially available reagents. To this end, Pall NAB plates were used in combination with reagents from two commercially available kits for gDNA preparation and two kits for RNA preparation. In addition, RNA was also prepared with the Pall NAB plate using a previously published protocol employing Standard Reagents[4]. For the RNA experiments, the latter served as a reference group.

The results show that the Pall NAB plates can be used with commercially available reagents of different manufacturers to deliver genomic DNA or RNA preparations with quality and yields similar to or better than obtained with the corresponding manufacturer's plate. For RNA, we found that both yield and quality of the reference group samples prepared according to an earlier published protocol compared favorably to the values obtained for samples prepared with the commercial reagents using either the corresponding commercial plates or the Pall NAB filter plate. In addition, we found that the average recovered eluate volumes obtained with the Pall NAB plate were higher than those obtained with the commercially available plates. This could be especially beneficial when processing samples anticipated to have low yields of RNA or genomic DNA. The improved elution efficiency allows use of lower elution volumes with the aim of increasing final sample concentrations.

2. Materials and Methods

Cell Culture

CHO/dhFr- cells (ATCC) were maintained in Minimal Essential Medium Alpha with GlutaMAX◆ Supplement and no nucleosides (Thermo Fisher Scientific) with added 1% Fetal Bovine Serum (Thermo Fisher Scientific), 1x HT (Sigma), and Penicillin (100 I.U./mL), Streptomycin (100 µg/mL) (Corning). The cells were kept in roller bottles (Corning) rotating at 4 rpm at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Cell concentration and viability were determined using a hemocytometer (Hausser Scientific) after diluting the cells 1:1 with 0.4% trypan blue (Amresco). After appropriate dilution in PBS, cells were transferred to a MASTERBLOCK◆ 96-well polypropylene storage plate with 1 mL well volume (Greiner Bio-One) at densities ranging from 0.5 to 5 × 10^6 cells/well for DNA preparations or 3.125 to 400 × 10^3 cells/well for RNA preparations. Cells then were pelleted by centrifugation for 5 min at 300 × g, after which the supernatant was removed by careful aspiration.

Genomic DNA Purification

Purification of genomic DNA from the freshly pelleted CHO cells with commercial reagents Com1 and Com2 were carried out as per manufacturers' instructions. For these experiments, DNA was eluted with a volume of 200 µL/well.

RNA Purification

For Isolation of RNA with Standard Reagents, lysis of freshly pelleted CHO cells was achieved by addition of 150 µL of GTC Lysis Buffer (Thermo Fisher Scientific; 4 M guanidine isothiocyanate, 50 mM Tris-HCl [pH 7.5], 25mM EDTA) to each well of the microplate, followed by vigorously shaking the plate back and forth while keeping the microplate flat on the bench. One volume (150 µL) of 70% ethanol was added to the lysate and mixed thoroughly after which the entire volume was transferred to wells of the Pall AcroPrep Advance 96-Well Filter Plates for Nucleic Acid Binding. RNA was allowed to bind to the media by vacuum filtration at 50.8 kPa (15 in Hg). Two washes were carried out by adding 170 µL/well RNA wash buffer (60 mM potassium acetate, 10 mM Tris-HCl [pH 7.5], 60% ethanol) followed by vacuum filtration to clear buffer. DNase digestion was performed while RNA was bound to the membrane by pipetting 80 µL/well of 0.5 U/µL DNase I (Thermo Fisher Scientific) in 40 mM Tris [pH 7.5], 10 mM NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$ onto the membrane and subsequently incubating it at room temperature for 20 min. The DNase was removed by two washes with 170 µL/well GTC Wash Buffer (0.2X GTC Lysis Buffer) followed by vacuum filtration to clear the buffer. Four additional washes were performed with 170 µL/well RNA Wash Buffer followed by vacuum filtration to clear buffer. The plate was then centrifuged at 1,500 × g until dryness for 2 min. RNAse free water (100 µL/well) was added and incubated for at least 1 minute after which RNA was eluted by centrifugation.

Purification of RNA with commercial reagents Com1 and Com2 were carried out per manufacturers' instructions. For these experiments, RNA was also eluted with 100 µL/well RNAse free water.

Evaluation of gDNA and RNA Samples - Concentration, Yield, and Quality

Genomic DNA and RNA concentrations were determined spectrophotometrically by measuring the absorption at 260 nm. The receiver plates were weighed pre- and post-elution and the weight difference was used to calculate the average recovered eluent volume per well. This average eluent well volume was then used to calculate the yield. Quality of the samples was determined by calculating the A_{260}/A_{280} ratio. Student's *t*-test was performed to discern differences in yield and quality. Groups were considered to differ significantly for P values < 0.05.

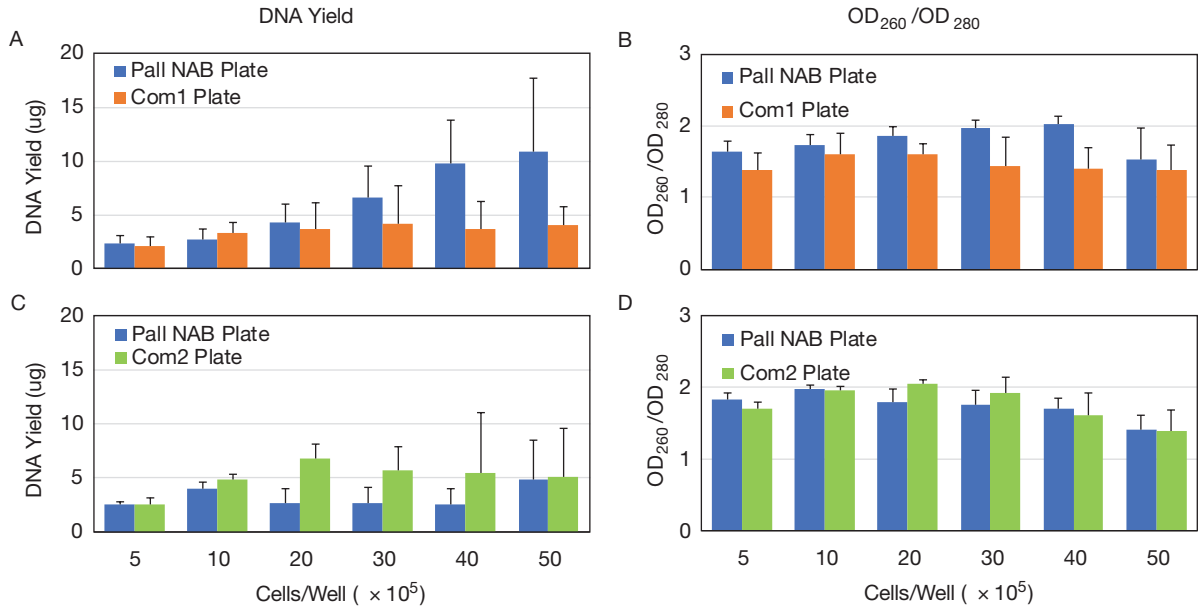
3. Results and Discussion

Genomic DNA Isolation from CHO cells

To investigate the broad versatility of the Pall NAB filter plate for isolation of genomic DNA from cultured mammalian cells with reagents from different manufacturers, genomic DNA was isolated from Chinese Hamster (*Cricetulus griseus*) Ovary (CHO) cells and evaluated in terms of yield and quality with the reagents from two commercially available kits for genomic DNA isolation using the Pall NAB filter plate and the corresponding commercial plates. Figure 1 shows the results of genomic DNA isolations from CHO cells at cell densities ranging from 0.5 to 5 × 10^6 cells/well. The resulting DNA yields are depicted in Figure 1, panels A and C. The quality of the preparations was assessed by determining the A_{260}/A_{280} ratios (panels B and D). This quality assessment is based on the fact that nucleic acids have absorbance maxima at 260 and 280 nm. The ratio of the absorbances at these wavelengths is commonly used to assess quality of nucleic acid extractions where ratios of ≥1.8 for DNA and ≥2.0 for RNA indicate that preparations are of good quality.

Figure 1

Yield and quality of genomic DNA isolated from CHO cells



Genomic DNA was isolated from CHO cells with commercial reagents (Com1 reagents in Panels A and B; Com2 reagents in Panels C and D) using the Pall NAB plate or using the corresponding commercially available plates (Com1 plate in Panels A and B; Com2 plate in Panels C and D). Panels B and D present the average A260/A280 ratios of the isolated DNA samples as a measure of DNA quality. The quality of DNA obtained with the Pall NAB plate with commercial reagents was better than or equal to that obtained with the corresponding commercial plates. Bars indicate averages of 16 samples. Error bars indicate the standard deviation.

Average DNA yields obtained with the Pall NAB plate using commercial reagents Com1 and Com2 tended to be similar to those with the corresponding commercial plates. However, with Commercial Plate 1, more wells clogged starting at cell densities as low as 2 × 10^6 cells/well. With the Pall plate, no clogged wells were observed at cell densities lower than 4 × 10^6 cells/well (data not shown).

For DNA prepared with Com1 reagents, the samples prepared with the Pall NAB plate tended to be of better quality (higher A_{260}/A_{280} ratios) than those prepared using the commercial plate, with exception of the samples at 1×10^6 cells/well, where quality could not be distinguished significantly ($P < 0.05$). No difference in quality was observed between the commercial plate and the Pall NAB plate for DNA prepared with Com2 reagents.

In all experiments, DNA was eluted with 200 µL/well buffer. Table 1 shows the average recovered eluate/well volumes. The average recovered elution volume of the Pall NAB plate was higher than that of plates Com1 and Com2. This property may allow more complete recovery when trying to elute with smaller volume in attempts to increase the eluent DNA concentration for instance when isolating DNA from low cell number samples.

Table 1

Average recovered elution volumes of genomic DNA samples

Filter Plate	Average Elution Volume (µL) Input	Recovered
Pall	200	200
Com1	200	168
Com2	200	185

RNA Isolation from CHO cells

The broad versatility of the Pall NAB plate for isolating RNA using reagents from different manufacturers was investigated following a similar approach as described above for genomic DNA. Here, RNA was isolated from Chinese Hamster Ovary (CHO) cells with reagents from two commercially available kits for RNA isolation using both the Pall NAB filter plate and the corresponding commercial plates. The results were compared against RNA isolated with the Pall NAB plate using a previously published protocol with Standard Reagents[2]. The resulting preparations were evaluated in terms of total yield and quality (A_{260}/A_{280} ratio).

Figure 2 shows the results of RNA isolations from CHO cells with Standard Reagents (Panels A and B) and with commercial reagents (Com3 reagents in Panels C and D; Com4 reagents in Panels E and F) using the Pall NAB plate or using the corresponding commercially available plates (Com3 plate in Panels C and D; Com4 plate in Panels E and F).

The yield of samples prepared with Com3 reagents using the corresponding commercial plate Com3 proved highly variable due to wells clogging and media in the wells becoming dislodged, which led to a loss of approximately 28% of the samples. The data presented for this group is derived from the wells which allowed recovery of RNA. The high variability in yields of samples that were obtained impeded meaningful statistical comparison with other groups.

Use of the Com3 reagents with the Pall NAB plate resulted in RNA yields that were grossly comparable to those obtained with Standard Reagents. When comparing the samples prepared with the Pall NAB plate using Com3 reagents (Panel C) to those prepared using Standard Reagents (Panel A), RNA yields were grossly comparable, except at lower cell numbers of up to 50×10^3 cells/well, where slightly higher yields were obtained using Standard Reagents ($P<0.05$).

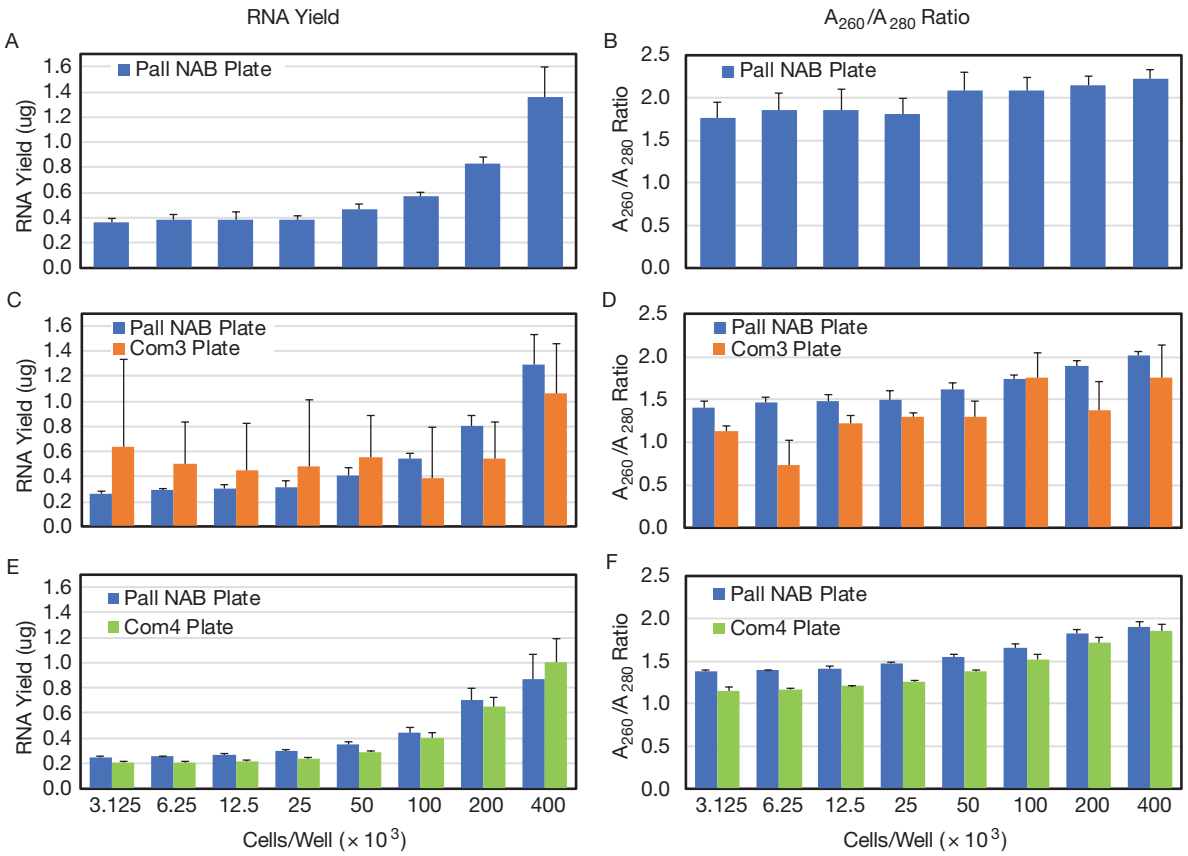
Use of Com4 reagents led to lower yields. When compared to the Pall NAB plate with Standard Reagents, RNA yields were reduced both for samples prepared with the Pall NAB plate (reduction 15 - 36%) and for the commercial plate (reduction 22 - 47%) ($P < 0.05$). For cell densities of up to 100×10^3 cells, RNA yields of samples prepared using the Pall NAB plate were higher than those of samples prepared with the Com4 plate ($P < 0.05$). At cell numbers higher than 100×10^3 cells, no significant differences were observed.

Quality of the RNA samples as determined by the A_{260}/A_{280} ratio is presented in panels B, D, and F of Figure 2. Samples of good quality have an A_{260}/A_{280} ratio ≥2.0. Samples prepared using Standard Reagents with the Pall NAB plate (panel B) appeared more uniform with A_{260}/A_{280} ratios ranging from 1.76 to 2.22 and were of better quality (higher A_{260}/A_{280} ratios) than the other preparations with commercial reagents ($P<0.05$).

Of the samples isolated using commercial reagents Com3 and Com4, those prepared with the Pall NAB plate tended to be of better quality than those prepared with the corresponding commercial plates ($P<0.05$), with exception of samples at the highest cell concentration of 400 × 10^3 cells, which could not be distinguished. In addition, for Com3 reagents the sample at 6.25 × 10^3 cells were of similar quality. The increased variability that can be observed in the quality of RNA obtained with the Com3 plate likely is due to the clogging and loose media problems encountered during preparation.

Figure 2

Yield and Quality of RNA isolated from CHO Cells



RNA was isolated from CHO cells with Standard Reagents (Panels A and B), or with commercial reagents (Com3 reagents in Panels C and D; Com4 reagents in Panels E and F) using the Pall NAB plate or using the corresponding commercially available plates (Com3 plate in Panels C and D; Com4 plate in Panels E and F). The quality of the RNA samples as determined by the A_{260}/A_{280} ratio is depicted in Panels B, D, and F. Bars indicate averages of 12 samples. Error bars indicate the standard deviation.

All sample elutions took place with 100 µL/well RNase free water aliquots. Table 1 shows the average recovered eluate/well volumes obtained with the tested plates. The average recovered elution volume of the Pall NAB plate was higher than that of plates Com3 and Com4. The higher proportion of the input volume that can be recovered following elution could be especially important when trying to elute with low volumes performed to increase the eluent RNA concentration.

Table 2
Average recovered elution volumes of RNA samples

Filter Plate	Average Elution Volume (µL) Input	Recovered
Pall	100	89
Com3	100	63
Com4	100	85

4. Summary

- Pall NAB plates can be used with a variety of reagents for gDNA and RNA applications. Quality and yield obtained with the Pall NAB plate were similar to or better than those obtained with the corresponding manufacturer's plate.
- Yield and quality of the reference group samples prepared according to earlier published protocols compared favorably to values obtained for samples prepared with the commercial reagents using either the corresponding commercial plates or the Pall NAB filter plate.
- Average recovered eluate volumes obtained with the Pall NAB plate were higher than those obtained with the tested commercially available plates. This could be especially beneficial using lower elution volumes with the aim of increasing sample concentration for samples from which only low amounts of RNA or genomic DNA are anticipated to be isolated.

5. References

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