PALL Life Sciences

Application Note

Genomic DNA Isolation From Whole Blood Using AcroPrep™ Advance 96-Well DNA Binding Plates on an Automated Liquid-Handling Robot

Introduction

DNA binding plates are employed as a basis for automated DNA isolation from blood for a broad variety of diagnostic tests. Such assays are used for disease detection, prognosis, and monitoring. Although these tests often utilize the same well-established automation platforms as basic research, they require a higher degree of robustness and sample consistency in terms of DNA yield, concentration, and quality.

There are a number of product options available in the market containing DNA binding plates and a complete set of buffers. Although vacuum filtration is more user-friendly and easily automated, centrifugation is still the primary method used to ensure DNA recovery and quality. An alternative to pre-packaged kits is the use of individual components, such as Pall AcroPrep Advance DNA binding plates which utilize standard glass fiber technology for genomic DNA isolation and purification. This platform offers additional flexibility as it can be used with a variety of reagent kits and, therefore, customized to your application requirements. Additionally, these plates offer a consistent and reproducible isolation of high yield and good quality gDNA from human blood using either vacuum or centrifugation.

The following protocol represents an example of a fully automated process for high throughput sample preparation from human blood (anticoagulated) optimized for the AcroPrep Advance DNA binding plates, and using commercially available buffers with the Eppendorf epMotion◆ 5075 liquid handling system.

Materials and Methods

Materials Required

- Pall AcroPrep Advance DNA binding filter plate (PN 8132)
- epMotion 5075 liquid handling system (Eppendorf)
- Eppendorf DWP (0030 522.109, 2000 µL/well)
- UV/Vis sample collection plate, capacity > 250 µL/well (Corning)
- Thermo adapter DWP 96 (Eppendorf 960002391)
- E-Gel◆ Agarose Gel Electrophoresis System (Invitrogen)
- E-Gel 48 1% Agarose gels (Invitrogen G8008 -01) and DNA markers for analysis of pure gDNA sample
- Quant-iT◆ Pico Green◆ dsDNA Assay Kit (Invitrogen P7589) or equivalent

Schematic of epMotion 5075 System Customized for DNA Isolation Protocol

Clamp	1000 µL 50 µL Pipettes	50 µL Tips	1000 µL Tips	Shaker/Heating Thermo Adapter DWP 96 (960002391)
	2 mL Proteinase K	Tub 1-5	Tub 6-7	Vacuum
Waste Bucket	Sample Prep Plate	Corning UV/Vis 96-well Plate	2 mL DNA Binding Plate	Vacuum Holder

Solutions Required

- AL lysis buffer (Qiagen 19075)
- Proteinase K, 20 mg/mL (Qiagen 19131)
- Wash buffers AW1 and AW2 (Qiagen PN 19081 and PN 19072, both required)
- Elution buffer (Qiagen 19077)
- 100% EtOH

Methods – Lysate Preparation and Purification Using Qiagen Buffers

Automation Set-Up

1. Place 2 mL Proteinase K in microfuge tube.
2. Place 20 mL of whole blood in tub 1.
3. Place 20 mL of 1x PBS in tub 2.
4. Place 20 mL of lysate buffer in tub 3.
5. Place 20 mL of 100% EtOH in tub 4.
6. Place 20 mL of elution buffer in tub 5.
7. Place 60 mL of wash buffer AW1 in tub 6.
8. Place 60 mL of wash buffer AW2 in tub 7.
9. Place appropriate collection and sample prep plates in epMotion vacuum.

Sample Prep Automation

**Pipette by each column (8 horizontal wells) and only change tips after each step to ensure shorter sample prep time.*

10. Add 20 µL Proteinase K into each well of sample prep plate.
11. Mix whole blood in tub 1 4x with pipette. Add 100 µL whole blood into each well.
12. Add 100 µL PBS into each well.
13. Mix sample 4x with pipette.
14. Add 200 µL buffer AL to each sample.
15. Mix sample 4x with pipette.
16. Shake sample prep plate at 1200 rpm for 3 minutes.
17. Incubate at 56 ºC (133 ºF) for 5 minutes. Shake plate for 1 minute at 1200 rpm. Incubate at 56 ºC (133 ºF) for 5 minutes.
18. Add 200 µL EtOH.
19. Mix 4x with pipette.
20. Shake sample prep plate at 1200 rpm for 3 minutes.

DNA Isolation Automation

21. Transfer 650 µL lysate mixture from sample prep plate to purification plate (Pall PN 8132) and place onto epMotion vacuum manifold.
22. Apply vacuum at 33 kPa for 10 minutes.
23. Wash with 700 µL/well buffer AW1.
24. Vacuum at 33 kPa for 10 minutes.
25. Wash with 700 µL/well buffer AW2.
26. Vacuum at 33 kPa for 10 minutes.
27. Remove Eppendorf DWP 2 mL collection plate.
28. Apply vacuum once more to ensure removal of residual alcohol ~5 minutes.
29. Place UV/Vis collection plate under Pall DNA binding plate.
30. Add 200 µL/well buffer AE into each well.
31. Incubate 2 minutes.
32. Apply vacuum at 33 kPa for 10 minutes. Tap DNA binding plate to ensure droplet collection. Collect gDNA.

gDNA Evaluation

33. Run 5 µL of each sample on 1% Agarose gel to assess quality and concentration.
34. Measure $OD_{260/280}$ of 50 µL of sample in UV compatible plates to determine concentration and purity.
35. Measure concentration of samples using Quant-iT Pico Green dsDNA assay kit.

Results

Experimental Design

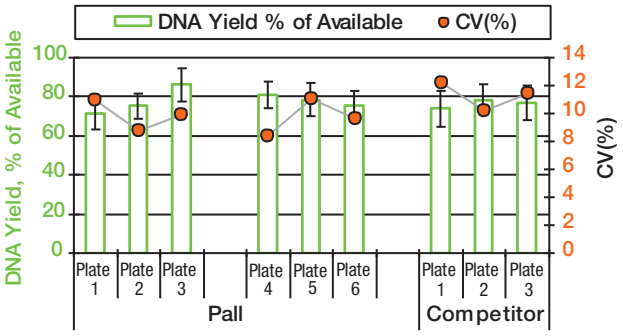
gDNA isolation from samples of fresh whole blood using AcroPrep Advance DNA binding plates and an alternative plate supplier was performed using the Qiagen gDNA buffer kit on the epMotion 5075 liquid handling system. The protocol was verified for DNA isolation from blood under different storage conditions: 1) fresh EDTA blood, up to 5-hour storage

at room temperature; 2) blood lysate stored for 5 hours before isolation begins; and 3) frozen/thawed blood. DNA yield (µg/well) from 100 µL of blood was measured by OD_{260} and Pico Green assays. Percent DNA recovery was calculated from the white blood cell concentration in each starting blood sample. DNA quality was assessed by $OD_{260/280}$ ratio for each well and gel electrophoresis was run for every other well in the plate. The robustness of the protocol was demonstrated using multiple plates from both manufactures. Intra- (well-to-well) and inter-plate (plate-to-plate) consistency was evaluated by comparing the averages, standard deviations (SD), and coefficients of variation [CV (%)] of each plate. Plates were also compared by their means and the upper and low limits of 95% confidential intervals. Six plates from Pall and three plates from a competitor were used in this study.

Pall vs. Competitor DNA Binding Plates
DNA Yield, Intra- and Inter-Plate Consistency

Samples of fresh blood (EDTA) produced the best gDNA yield at 3.7 µg/well (100 µL blood). That represents up to 86% DNA recovery calculated based on WBC concentration in the starting blood sample. Inter-plate performance for DNA yield was similar for all nine plates used in the study varying in the range of one SD between 79-86%. Intra-plate (well-to-well) consistency evaluated by CV (%) was 11% or less. Data are presented in Figure 1. The AcroPrep Advance plates demonstrated performance similar to or better than the competitor's plates when used with the Qiagen gDNA buffer kit.

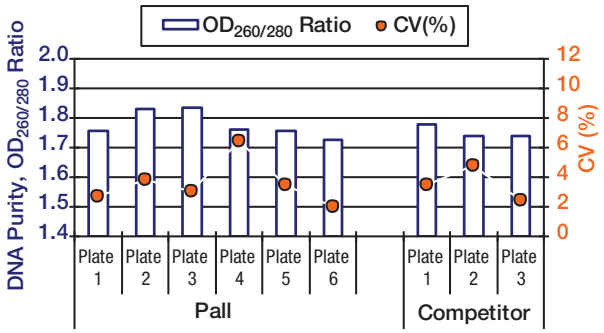
Figure 1
Pall vs. Competitor's DNA Binding Plates: DNA Yield, Intra- and Inter-Plate Consistency



DNA Purity as $OD_{260/280}$ Ratio: Intra- and Inter-Plate Consistency

Inter-plate performance for $OD_{260/280}$ ratio was similar for all nine plates used varying in the range of one SD between 1.72-1.83 with intra-plate consistency CV (%) of 6% or less. This performance is superior to that which is commonly expected for the application. Data are presented in Figure 2. The Pall plates produced high quality DNA similar to that produced by the competitor's plates.

Figure 2
Pall vs. Competitor's DNA Binding Plates: DNA Purity as $OD_{260/280}$ Ratio, Intra- and Inter-Plate Consistency

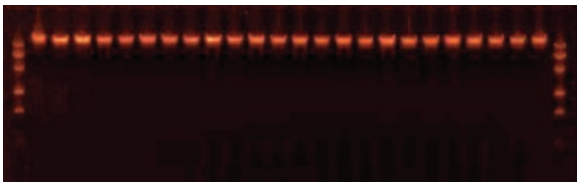


Consistency of Performance: DNA Gel Electrophoresis

Figure 3 provides images of gel electrophoresis runs for every other well from the 96-well plates from Pall (A) and the competitor (B). The results for 24 wells are presented. Similar intensity of fluorescence and uniform positioning of the signal at a large molecular weight area indicates high quality and similar DNA concentration in all wells.

Figure 3
Gel Electrophoresis of DNA Isolated Using Pall Plate (A) and Competitor's Plate (B)

A – Pall Plate



B – Competitor's Plate

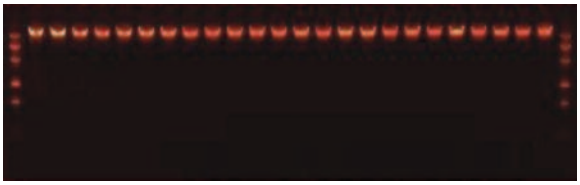


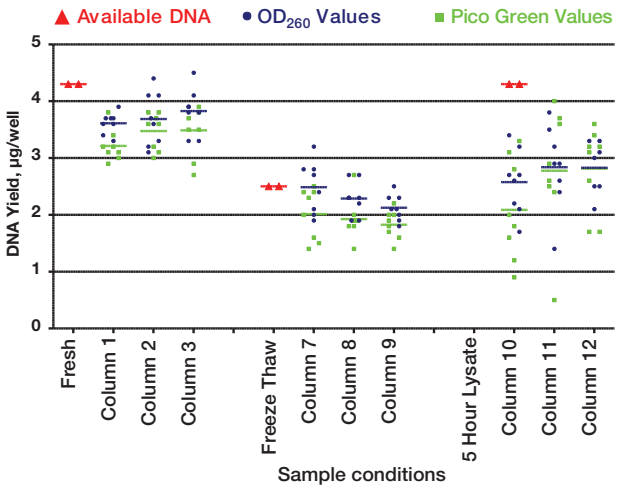
Plate Performance with Blood Sample Under Different Storage Conditions

The protocol was verified for DNA isolation from blood under three different storage conditions: 1) fresh EDTA blood stored for up to five hours at room temperature; 2) blood lysate stored for five hours before isolation; and 3) frozen and thawed blood. Fresh blood produced the best gDNA yield at 3.7 µg/well and 86%

total recovery. Five-hour lysate blood produced poor gDNA yield at 2.7 µg/well and 63% total recovery. Freeze/thaw blood demonstrated a decrease in WBC number and in DNA yield from 3.7 to 2.3 µg/well compared to the fresh sample. The purity of gDNA for all conditions was in the range of industry expectations – $OD_{260/280}$ ratio of 1.7-1.9 from fresh blood and 1.6-1.8 from 5-hour lysate and freeze thaw blood. Data are presented in Figure 4.

Figure 4

Pall Plate Performance with Blood Under Different Blood Sample Conditions: DNA Yield (µg/Well)



Each blood storage condition is presented by 24 individual data points and averages. Red triangles show the value of expected DNA yield calculated based on the WBC concentration in the sample.

Discussion

DNA can help diagnose or indicate a susceptibility to having or passing on a wide range of diseases, such as cystic fibrosis, Huntington's disease, sickle cell anemia, and Tay-Sachs. Fresh or frozen blood is the most common specimen used for this type of analysis, and sample preparation is considered one of the more critical factors in achieving the levels of sensitivity and specificity required. Fast and efficient DNA isolation from whole blood is essential to obtain the necessary high quality starting material.

Many research labs achieve high levels of DNA sample yield, concentration, and quality while obtaining fast and efficient sample preparation through the use of complete kits that include binding plates and buffers. Pall offers an alternative to this approach by selling DNA binding plates as a separate component that can be used with the solution kits of many manufacturers. A key advantage is that the total cost of the Pall DNA binding plates and buffers can be 50% of the cost of the complete kits that incorporate binding plates and buffers. Additionally, such an approach offers added flexibility for your specific requirements, and the versatility to purify samples using either vacuum or centrifugation.

The purpose of this study is to compare the DNA recovery and purity of samples prepared with the AcroPrep Advance DNA binding plate and popular commercial buffers to samples prepared with a competitor's plate. The two different plates were used with the same materials and methods based on vacuum filtration and using a liquid-handling robotic system popular in research facilities.

The results show that DNA recovery is similar for the Pall plates and the competitor's plates. The six Pall plates ranged from 71-86% DNA recovery, while the three competitor's plates ranged from 73-79% recovery. Intra-plate (well-to-well) consistency was also similar for all nine plates with CV (%) of 11% for all nine plates, indicating that the purification process is highly reproducible. Inter-plate CV (%) was also similar for all nine plates with intra-plate consistency CV (%) of 6% or less.

DNA purity is also at similar high levels for both Pall's and the competitor's plates. The $OD_{260/280}$ ratio was in the range of between 1.73-1.83 for the six Pall plates and was between 1.72-1.79 for the three competitor's plates. The inter-plate consistency for DNA purity was in the range of one SD for all nine plates in the study with intra-plate consistency CV (%) within 6% or less.

Gel electrophoresis images indicate similar levels of quality and DNA concentration on the samples produced with both the Pall binding plates and the competitor's plates.

The study also clearly demonstrates the advantages of using fresh blood samples in research studies. Fresh blood produced the highest yields at 3.7 µg/well. Blood lysate that had been stored for 5 hours produced a lower DNA yield of 2.7 µg/well. Blood that had been frozen and thawed provided an even lower DNA yield of 2.3 µg/well.

These results demonstrate that AcroPrep Advance DNA binding plates can be used in conjunction with commercially available buffers to deliver equal or better performance than a competitor's plate. The combination of the AcroPrep Advance DNA binding plate and commercial buffers provides a 50% cost savings compared to the purchase of a complete kit. This study demonstrates that research labs can achieve a substantial consumable cost savings in sample preparation without sacrificing DNA yield, purity, or quality.

Summary

- AcroPrep Advance DNA binding plates provide equal or better performance than competitor's plates when used with commercially available buffers.
- AcroPrep Advance DNA binding plates plus buffers cost about 50% less than competitor's complete kit including binding plates and buffers.
- DNA yield and purity of Pall's DNA binding plates is equal to or better than competitor's plates.
- Inter-plate and intra-plate consistency for both DNA yield and purity of the samples produced with the Pall plates is equal to or better than the competitor's plates.
- Gel electrophoresis images indicate high quality and similar DNA concentration for both Pall and competitor plates.
- Fresh blood provides the highest DNA yields while blood lysate stored for 5 hours and freeze/thawed blood provide substantially lower yields.

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