Technical Note

PALL®

Lysate Clearance

Introduction

Molecular biology methods have led to the successful production of a variety of synthesized biomolecules (DNA, RNA, proteins) from prokaryotic and eukaryotic cell lines. The most problematic step in the purification of these biomolecules is the clarification of the sample once the cells are lysed. The lysate often contains biomolecule concentrations millions of times higher than the molecule of interest.

In the past, centrifugation was the primary method used to sediment cellular debris. Sedimentation has several limitations for many applications where small-scale, high-throughput processing is required.

Filtration, which is easily automated, is relatively quick and allows the use of additional wash steps to maximize sample recovery. Filtration can be done effectively in either vacuum or centrifugal mode, ultimately maximizing the choice in protocols available to the researcher.

We present a lysate sample preparation procedure using the AcroPrep™ Advance filter plate for lysate clearance that effectively removes unwanted cellular debris from samples.

Consumables

- AcroPrep Advance filter plate for lysate clearance, 3.0 µm glass fiber/0.2 µm Supor® membrane, PN's 8075 (350 µL), 8175 (1 mL), or 8275 (2 mL) http://www.pall.com/main/laboratory/product.page?lid=gri78lvo
- Pall multi-well plate vacuum manifold, PN 5017 http://www.pall.com/main/laboratory/product.page?lid=gri78lvo
- Pall adapter collar for centrifugation, PN 5225 http://www.pall.com/main/laboratory/product.page?lid=gri78lvo
- VWR◆ deep well microplates, 2 mL, VWR PN 40002-014
- Corning Axygen◆ 96-well retention plate, PN P-DW-20-C-S (2 mL) or P-2ML-SQ-C-S (2.2 mL)
- Thermo Fisher Scientific Invitrogen◆, One Shot◆ MAX Efficiency◆ DH5α◆-T1R Competent Cells, PN 12297-016

Solutions

- Luria-Bertani (LB) media
- Resuspension buffer (50 mM Tris-Cl pH 8.0, 10 mM EDTA, 100 µg/mL RNase A)
- Lysis buffer (200 mM NaOH, 1% SDS)

Protocol

For clearing lysate of *E. coli* containing DNA plasmids.

Culture growth and lysis

From flasks

a. Grow *E. coli* containing pCAT plasmid cultures in LB + ampicillin overnight at 37 °C.

b. Split cultures into 50 mL aliquots.

c. Centrifuge aliquots to pellet cells down.

d. Remove supernatant by aspiration.

e. Resuspend each pellet in 5 mL of resuspension buffer.

f. Combine 2 aliquots of resuspended cells for a final volume of 10 mL.

g. Add 10 mL of lysis buffer to 10 mL of resuspended cells.

h. Invert tube 2-3 times to mix.

i. Add 10 mL of neutralizing buffer.

j. Invert tube 2-3 times to mix.

k. Store on ice for 5 minutes.

From microplates

a. In each well of deep well microplate, grow 1 mL of *E. coli* containing pCAT plasmid cultures in LB + ampicillin overnight at 37 °C with shaking.

b. Spin down culture plate for 10 minutes.

c. Aspirate media.

d. Resuspend each pellet in 100 µL resuspension buffer.

e. Add 100 µL of lysis buffer to each well.

f. Shake microplate for 2 minutes.

g. Add 100 µL of neutralizing buffer to each well.

h. Shake microplate for 2 minutes.

Transfer flocculent lysate to wells of an AcroPrep Advance filter plate for lysate clearance

Proceed to clarification of lysate through vacuum or centrifugal filtration

With vacuum filtration

- Place collection plate in a Pall multi-well plate vacuum manifold.
- Place filled lysate clearance plate on top of the vacuum manifold.
- Apply vacuum (10 in. Hg) and start filtration.
- Release vacuum.

With centrifugal filtration

- Put adapter collar for centrifugation on receiver plate.
- Stack filled lysate clearance plate on top of receiver plate.
- Place stacked plates in a standard swinging bucket microtiter plate rotor assembly.
- Centrifuge. As a general guideline, centrifugation at 1,500 x g for 1 to 2 minutes is sufficient to evacuate the well contents.

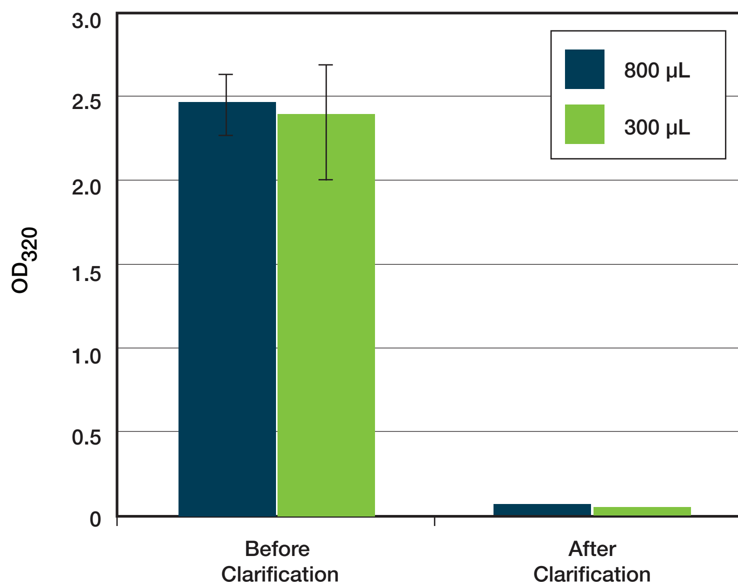
Discard lysate clearance filter plate

Retrieve receiver plate

Filtrate is now ready for downstream applications

Figure 1

Clarification Effectiveness as a Measure of Absorbance



OD320 measurement of flocculent lysate before and after filtration with AcroPrep Advance filter plate for lysate clearance. 300 µL is the standard volume of lysate obtained from 1 mL of culture.

Results and Discussion

To demonstrate the effectiveness of the AcroPrep Advance filter plate for lysate clearance, turbidity was measured before and after clarification. OD measurement is a good indicator of turbidity of samples.

Figure 1 shows that the turbidity of samples was reduced by more than 95% after filtration with the AcroPrep Advance filter plate for lysate clearance.

Once the lysate is clarified, we can proceed to downstream applications such as:

A. Purification of plasmid DNA with an Acroprep Advance filter plate for nucleic acid purification.

B. Desalting using an AcroPrep Advance filter plate for ultrafiltration.

C. RNA isolation with AcroPrep Advance filter plate for nucleic acid purification.

It should be noted that there were no instances of wells clogging during filtration.

The AcroPrep Advance filter plate for lysate clearance effectively removes cellular debris from samples prior to purification of biomolecules. It is a great tool that provides maximum biomolecule recovery for researchers doing small-scale high-throughput processing.

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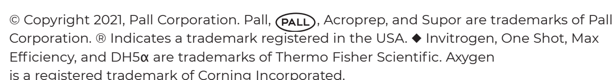
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