Optimizing the Purification of Tagged Proteins Using Multi-well Filter Plates

Tao Hu, Ph.D. Cold Spring Harbor Laboratory and Kevin Seeley, Ph.D. Pall Corporation

Abstract

Background

Sample Incubation

The following additional information is available from Pall Life Sciences:

- Desalting/Buffer Exchange for Biomolecules Using AcroPrep 96 Filter Plate with GHP Membrane, PN 33245
- Pall Life Sciences: 800-537-0600
- Life Sciences Brochure: 800-537-0600

Well-to-well Reliability Using Multi-well Microlinucors

Conclusions

The following references were used:

- Optimizing IMAC Metal and Resin Selection
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References

• Proprietary design minimizes solution/sample weeping
• Rigid single-piece construction
• Millipore

- Optimize resin-to-load ratio

- Chelating resin: Zn 2+, Cu 2+, Co 2+

- Elution buffer: 100 mM NaPi, 6M urea, pH 3.0

- Filter plate has the following advantages:

- Elution buffer: 100 mM NaPi, 6M urea, pH 3.0

- Buffer recommendations:

- AcroPrep 96 Filter Plate has the following advantages:

- The AcroPrep 96 filter plate is designed to fit most of the manual manifolds as well as robotic equipment. Combined with immobilized metal affinity resin, the plate can be used for the preparation of recombinant proteins isolated from IMAC resin minicolumns. The system is typically needed to purify biomolecules from crude lysates. This study shows that the use of multi-well filter plates allows the simultaneous testing of different elution conditions.

- The slurries were incubated in the individual wells of an AcroPrep 96 Bio-Inert 0.2 µm filter plate (PN 5042) for 30 minutes at 4 ºC. After washing the resin twice with 200 µL of 6M urea, 100 mM NaPi, the samples were eluted using three elution volumes of 200 µL each. The flow through, wash and elution fractions from each well were analyzed on a 4 to 12% SDS PAGE gel. The use of multi-well filter plates allows the simultaneous testing of different elution conditions for purifying different proteins. Well-to-well testing using multi-well minicolumns can help determine the optimal elution pH for each protein.

- Zn 2+ charged resin performed well in purifying the tagged protein. Our data indicate that the resin-to-load ratio is another critical factor to ensure the optimal elution of the tagged protein. The information obtained in minicolumn can help determine the optimal elution pH for each protein.

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- The elution from each well was analyzed on a 4 to 12% SDS PAGE gel. The protein concentration of the eluted protein from each well was determined by BCA assay. The high-throughput sampling procedure demonstrates the potential for routine recovery of tagged proteins from a simple and robust purification procedure.

- Ninety-six 20 µL aliquots of Ni-NTA resin (Qiagen) were mixed with 100 µL of an equal volume of sample to be tested. The slurries were incubated in the individual wells of an AcroPrep 96 Bio-Inert 0.2 µm filter plate (PN 5042) for 30 minutes at 4 ºC. After washing the resin twice with 200 µL of 6M urea, 100 mM NaPi, the samples were eluted using three elution volumes of 200 µL each. The flow through, wash and elution fractions from each well were analyzed on a 4 to 12% SDS PAGE gel. The use of multi-well filter plates allows the simultaneous testing of different elution conditions for purifying different proteins. Well-to-well testing using multi-well minicolumns can help determine the optimal elution pH for each protein.

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