Simple protein purification with magnetic beads
- from low µL to high mL

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Content

Introduction – Protein purification with Mag Sepharose™

Low sample volume
- Reproducibility
- Immunoprecipitation
- Histidine-tagged protein

Large sample volume
- Purification and concentration of large volumes of low expression protein

Membrane protein purification with magnetic beads

Summary
Content

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Summary
# Formats for protein purification and enrichment

<table>
<thead>
<tr>
<th>Format</th>
<th>Microcentrifuge</th>
<th>Centrifuge/vacuum</th>
<th>System/syringe</th>
<th>No special equipment</th>
<th>Magnetic device</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SpinTrap™</strong></td>
<td>µg-mg scale</td>
<td>Fixed amount of beads</td>
<td>Fixed maximum sample volume</td>
<td>Automatable</td>
<td></td>
</tr>
<tr>
<td><strong>MultiTrap™</strong></td>
<td>µg-mg scale</td>
<td>Fixed amount of beads</td>
<td>Fixed maximum sample volume</td>
<td>Fixed maximum sample volume</td>
<td>Automatable</td>
</tr>
<tr>
<td><strong>HiTrap™</strong></td>
<td>µg-mg scale</td>
<td>Fixed amount of beads</td>
<td>Variable maximum sample volume</td>
<td>Fixed maximum sample volume</td>
<td></td>
</tr>
<tr>
<td><strong>GraviTrap™</strong></td>
<td>µg-mg scale</td>
<td>Fixed amount of beads</td>
<td>Variable maximum sample volume</td>
<td>Fixed maximum sample volume</td>
<td></td>
</tr>
<tr>
<td><strong>MagSepharose™</strong></td>
<td>Low µg scale-mg scale</td>
<td>Variable amount of beads</td>
<td>Variable sample volume</td>
<td>Automatable</td>
<td></td>
</tr>
</tbody>
</table>

![Images of different formats](image1.png)
Introduction – Protein purification with Mag Sepharose™

- Efficient, high capacity small-scale purification/screening of antibody and histidine-tagged proteins
- Simple capture of target proteins
- Works from low microlitre to high millilitre sample volumes
Introduction – Protein purification with Mag Sepharose™

Equilibration → Binding → Washing → Elution
Content

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Summary
Reproducibility

- Binding of human IgG to protein G Mag Sepharose™ Xtra down to 1 µL beads

**Experiment**

<table>
<thead>
<tr>
<th>Amount beads (µl)</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2</th>
<th>1</th>
<th>No beads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount loaded IgG (µg)</td>
<td>100</td>
<td>80</td>
<td>60</td>
<td>40</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

**Sample**  Human IgG diluted to 0.5 mg/ml  
**Media**  Protein G Mag Sepharose Xtra  
**Binding buffer**  PBS  
**Elution buffer**  100 mM glycine-HCl, pH 2.8  

The experiment was carried out in a 96-well plate and done automatically on a Tecan robot.
Reproducibility

- Binding of human IgG to protein G Mag Sepharose™ Xtra down to 1 µL beads

Results
Reproducibility

- Binding of human IgG to protein G Mag Sepharose Xtra down to 1 µL beads

Results

Robust and reproducible results
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Summary
Immunoprecipitation

**Work flow**

- Magnetic bead with protein A/G or NHS activated bead
- Binding or crosslinking or coupling of antibodies
- Binding of target protein
- Washing
- Elution
Immunoprecipitation
- Enrichment of plasminogen from human plasma with NHS Mag Sepharose™

Experiment:

Medium: 25 μl NHS Mag Sepharose 20% gel slurry
Sample: Human plasma
Sample volume: 150 μl
Antibody: Monoclonal mouse anti-plasminogen, subtype IgG1
Coupling buffer: 150 mM triethanolamine, 500 mM NaCl, pH 8.3
Binding buffer: TBS, 50 mM Tris, 150 mM NaCl, pH 7.5
Wash buffer: TBS, 2 M urea, pH 7.5
Elution buffer: 0.1 M glycine/HCl, 2 M urea,
Immunoprecipitation
- Enrichment of plasminogen from human plasma with NHS Mag Sepharose™

Results:

Start material

SDS-PAGE

1st and 2nd elution

1000 fold enrichment!
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Summary
Low sample volume
- Histidine-tagged protein

Stable peptide
Compatible with many chemicals
Functional under denaturing conditions

Immobilized Metal Affinity Chromatography
Chelating ligand attached to support
Metal ion (Ni$^{2+}$, Co$^{2+}$, Cu$^{2+}$)
Histidine-tagged protein
Mild elution conditions
Green Fluorescent Protein

$M_r \times 10^3 \text{Da}: \sim 27$

Originally isolated from a jellyfish, it exhibits bright green fluorescence, extremely useful in cell biology and other biological disciplines.

Expressed in *E. coli* with a $(\text{His})_6$-tag located on the C-terminus.
Low sample volume
– Purification of Histidine-tagged GFP with His Mag Sepharose\textsuperscript{TM} Ni

Experiment:

<table>
<thead>
<tr>
<th>Amount beads (µl)</th>
<th>5</th>
<th>4</th>
<th>3</th>
<th>2.5</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amount loaded GFP-His (µg)</td>
<td>94</td>
<td>75</td>
<td>56</td>
<td>47</td>
<td>38</td>
<td>19</td>
</tr>
</tbody>
</table>

Sample | GFP-(His)$_6$ spiked in \textit{E.coli} lysate

Media | His Mag Sepharose Ni

Bindning buffer | 20 mM Na phosphate, 500 mM NaCl, 20 mM imidazole pH 7.4

Elution buffer | 20 mM Na phosphate, 500 mM NaCl, 500 mM imidazole pH 7.4

16 replicates were done for each gel volume.
The experiment was carried in a 96-well plate.
Low sample volume
– Purification of Histidine-tagged GFP with His Mag Sepharose™ Ni

Results:

High reproducibility!

Eluted protein

SDS-PAGE
Content

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Summary
Large sample volume

- Purification of 50 mL low expressed Mouse IgG$_2$b on protein A Mag Sepharose$^\text{TM}$ Xtra

Experiment:

<table>
<thead>
<tr>
<th>Sample</th>
<th>monoclonal mouse IgG$_2$b (~ 0.07 mg/mL) in 50 ml diluted cell supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>175 µL bead of Protein A Mag Sepharose Xtra</td>
</tr>
<tr>
<td>Binding buffer</td>
<td>PBS</td>
</tr>
<tr>
<td>Elution buffer</td>
<td>100 mM glycine-HCl, pH 2.8</td>
</tr>
</tbody>
</table>

Purification performed in duplicate
Large sample volume

– Purification of 50 mL low expressed Mouse IgG$_{2b}$ on protein A Mag Sepharose$^{TM}$ Xtra
Large sample volume

- Purification of 50 mL low expressed Mouse IgG\textsubscript{2b} on protein A Mag Sepharose\textsuperscript{TM} Xtra

Results:

<table>
<thead>
<tr>
<th></th>
<th>Replicate 1</th>
<th>Replicate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>2.4 mg</td>
<td>2.4 mg</td>
</tr>
</tbody>
</table>

SDS-PAGE

1. LMW
2. Start material
3. Flow through, replicate 1
4. Flow through, replicate 2
5. Eluate replicate 1
6. Eluate, replicate 2
## Comparison

<table>
<thead>
<tr>
<th>Protein A Mag Sepharose™</th>
<th>Protein A Mag Sepharose Xtra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein G Mag Sepharose</td>
<td>Protein G Mag Sepharose Xtra</td>
</tr>
<tr>
<td><strong>Antibody binding capacity:</strong> +</td>
<td><strong>Antibody binding capacity:</strong> ++</td>
</tr>
<tr>
<td><strong>Optimized for Immunoprecipitation</strong></td>
<td><strong>Optimized for purification of antibodies</strong></td>
</tr>
</tbody>
</table>
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Summary
Membrane proteins
- Considerations

Expression
- Host cell
- Expression level
- Tag

Preparation
- Cell membranes
- Purification Strategy
  - Aim of purification
  - Detergents
  - Purification media
Membrane proteins
- Detergent solubilization

- Detergents
- Critical Micelle Concentration (CMC)

<table>
<thead>
<tr>
<th>Detergent</th>
<th>CMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauryldimethylamine -N-oxide (LDAO)</td>
<td>0.02%</td>
</tr>
<tr>
<td>Octyl glucoside (OG)</td>
<td>0.53%</td>
</tr>
<tr>
<td>Fos-Choline 12</td>
<td>0.05%</td>
</tr>
<tr>
<td>Decyl Maltoside (DM)</td>
<td>0.09%</td>
</tr>
<tr>
<td>Dodecyl Maltoside (DDM)</td>
<td>0.009%</td>
</tr>
<tr>
<td>CYMAL™-5</td>
<td>0.12%</td>
</tr>
<tr>
<td>Dodecyl octaethylene glycol ether (C₁₂E₈)</td>
<td>0.005%</td>
</tr>
</tbody>
</table>
Membrane proteins
- Detergent solubilization

Solubilisation
Mix Membranes + detergent stock solution. Incubate at room temperature for 30 min.

Clarification
Centrifuge at 50 000 x g.

Purification (IMAC)
Collect supernatants and add His Mag Sepharose™ Ni into each detergent solubilized membrane protein sample.

Analysis of target membrane protein
Evaluate purity and homogeneity.
Membrane proteins
- Detergent solubilization screening of YedZGFP-His

YedZGFP-His
Integral membrane-flavocytochrome with six transmembrane segments

Mr x $10^3$Da: ~ 40

Unknown function

Expressed in *E. coli* with a (His)$_8$-tag located on the C-terminus of the GFP part
Membrane proteins
- Detergent solubilization screening of YedZGFP-His

Experiment

Screen 7 detergents

900 µL of cell membranes solubilized in 100 µL 10% detergents for 30 min

Centrifugation at 50 000 x g for 30 min

Supernatant applied to the His Mag Sepharose™ Ni
  Binding buffer contained 40 mM imidazole

Evaluation
  SDS-PAGE; Deep Purple™ stain, GFP-fluorescence
Membrane proteins
- Detergent solubilization screening of YedZGFP-His

Results
Membrane proteins
- Scale-up from Magnetic beads to Column

YedZGFP-His using DM

Same preparation process as used in the Mag Sepharose™ scale
 Membranes solubilized in 1% DM
 Purification at 0.2% DM

25 mL of solubilized supernatant applied to a 1 mL HisTrap™ HP column
Membrane proteins
- Scale-up from Magnetic beads to Column

Blue curve = $A_{280}$
Red curve = $A_{490}$

FT Eluate

YedZGFP-His

Gel: Excel 8-18
Stain: Deep Purple™
Membrane proteins
- Magnetic beads vs column

<table>
<thead>
<tr>
<th>1 mL sample</th>
<th>25 mL sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>His MagSepharose™ Ni</td>
<td>HisTrap™ HP</td>
</tr>
</tbody>
</table>
Questions?
Summary

Magnetic Beads provides:

- Easy screening of target protein with high reproducibility
- Scalability: Simple capture of target protein from small (μL) to large (mL) sample volumes.
- Ideal for simple and rapid detergent screening/purification of membrane proteins.
- Useful information for larger scale preparations
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Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US pat 5,284,933 and US pat 5,310,663, including corresponding foreign patents (assignee: Hoffman La Roche, Inc).

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