Development of High Capacity Magnetic Protein A Beads and Protein G Beads for Rapid Antibody Purification, Characterization and Drug Conjugation

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1. Introduction
We have developed high capacity magnetic Protein A and magnetic Protein G beads by combining two novel technologies.
1. Use of magnetic beads based on high surface area macroporous cellulose
2. Covalent and oriented immobilization of Protein A and Protein G on the magnetic bead using HaloTag™ Technology

Magne™ Protein A Beads and Magne™ Protein G Beads purify polyclonal and monoclonal antibodies from serum, ascites fluid and cell media.

High capacity magnetic Protein G and Protein A beads can be used to conjugate antibodies with fluorescent dyes and small molecules for ADC applications.
Advantages of on-bead antibody conjugation includes, no need of purified antibodies, labeling through thiol or amine groups, efficient labeling of dilute antibodies, no dialysis or buffer exchange is required and parallel labeling of up to 96 samples.

2. Oriented and Covalent Attachment on Magnetic Beads

- HaloTag® is a 33 kDa protein fusion tag engineered to covalently bind a synthetic HaloTag® ligand
- Oriented Protein A or Protein G contribute to high capacity of the beads for antibody purification
- Oriented Protein G and Protein A tested on ForteBio Octet Biosensor show expected binding to antibody

3. Antibody Purification: Magne™ Protein A Beads and Magne™ Protein G Beads

<table>
<thead>
<tr>
<th>Species</th>
<th>Isotype</th>
<th>Magne™ Protein G Beads</th>
<th>Magne™ Protein A Beads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bovine</td>
<td>IgG</td>
<td>33</td>
<td>36.2</td>
</tr>
<tr>
<td>Goat</td>
<td>IgG</td>
<td>26</td>
<td>26.2</td>
</tr>
<tr>
<td>Human</td>
<td>IgG</td>
<td>36.4</td>
<td>34.8</td>
</tr>
<tr>
<td></td>
<td>IgG2</td>
<td>19.4</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>IgG2a</td>
<td>31.6</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>IgG2b</td>
<td>31.4</td>
<td>29.6</td>
</tr>
<tr>
<td></td>
<td>IgG3</td>
<td>30.4</td>
<td>18.6</td>
</tr>
<tr>
<td>Mouse</td>
<td>IgG</td>
<td>33.2</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td>IgG2a</td>
<td>31.6</td>
<td>33.2</td>
</tr>
<tr>
<td></td>
<td>IgG2b</td>
<td>31.4</td>
<td>29.6</td>
</tr>
<tr>
<td>Porcine</td>
<td>IgG</td>
<td>9.2</td>
<td>8.5</td>
</tr>
<tr>
<td>Ret</td>
<td>IgG2a</td>
<td>1.2</td>
<td>16</td>
</tr>
<tr>
<td>Sheep</td>
<td>IgG</td>
<td>3.2</td>
<td>29.4</td>
</tr>
</tbody>
</table>

50µg antibody in 1ml purified with 50µl bead slurry in triplicate (CV ≤10%)

Both Protein A and Protein G binds with high affinity to the Fc section of the antibody.
The binding affinity of Protein A and Protein G to antibody from different isotype and from different species differ significantly as shown in Table

Advantages of Magnetic Beads
- Simple and easy to use
- Several samples (1-96) can be handled in parallel
- Sample volumes of 20µl to 50µl
- Antibody can be eluted at high concentrations
- Purification can be automated

4. Scalable and Multiple Purifications using Magnetic Protein G and Protein A Beads

<table>
<thead>
<tr>
<th>Purification Scale (ml)</th>
<th>Protein G Beads (ml)</th>
<th>murine IgG2a purified (µg)</th>
<th>murine IgG2 purified (µg)</th>
<th>murine IgG1 purified (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.05</td>
<td>58</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>500</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>2.5</td>
<td>2910</td>
<td>980</td>
<td></td>
</tr>
</tbody>
</table>

- Antibody purification is scalable from 1.0ml to 50ml samples
- Antibody can be purified from sample as low as 20µl containing 10µg/ml antibody

5. Antibody Conjugation on Magnetic Beads

- Antibody drug conjugates need optimization of several parameters including linker length, drug to antibody ratio.
- High capacity magnetic Protein G/A beads are ideal for screening antibody conjugation in a single workflow without any need for purified antibody.

6. Antibody Conjugation through Lysines

- Human IgG was captured on the Protein G beads and labeled with AlexaFluor® 647 succinimidyl ester that binds to lysines on antibodies
- Labeling was done in 15min in borate buffer at pH 9.0

Advantages:
- Can purify and label small quantity of antibody
- Rapid conjugation of antibody with small molecules (Ex: Fluorescent ligand)
- Easy optimization of ligand and antibody concentrations
- Unreacted ligand washed from beads before antibody elution
- No antibody loss or dilution from dialysis to remove unreacted ligand
- Successfully label antibodies through amine and sulphydryl groups

7. Antibody Purification and Conjugation

- Three different mouse antibody isotypes from cell-media were captured on the beads and labeled using AlexaFluor® 647 succinimidyl ester
- Antibody labeling as a function of dye concentration for all three isotypes was done in parallel
- Antibody labeling directly from cell media can be done
- AlexaFluor®647 gel scan shows increasing labeling with increase in dye amount
- Commassie gel scan of same gel shows excellent antibody recovery

8. Antibody Conjugation through Thiolis

- Conjugating antibodies using selective reduction of thiol bonds in antibody hinge region is important for making antibody drug conjugate
- Human IgG was captured on the Protein G beads, reduced using 10mM TCEP at room temperature and 37°C for 30min and labeled with increasing amount of AlexaFluor®647 Maleimide.

Results
- Antibody as low as 100µg/ml can be labeled with excellent recovery
- Ligand to antibody ratio increases with the increasing antibody concentration and dye concentration

- Antibody labeling as a function of dye concentration for all three isotypes was done in parallel
- Antibody labeling directly from cell media can be done
- AlexaFluor®647 gel scan shows increasing labeling with increase in dye amount
- Commassie gel scan of same gel shows excellent antibody recovery

High capacity magnetic Protein G and Protein A beads: ≥ 25 mgs of Human IgG/ml of settled bead
- High purity and high recovery of monoclonal and polyclonal antibodies in small volumes
- Compatible with different species and different isotypes of antibodies in variety of sample types including cell culture, ascites and serum samples
- Scalable: sample volumes from 20µl to 50ml
- Throughput: 1-96 samples
- High capacity allows on-bead antibody conjugation using small molecules
- Ability to synthesize multiple antibody conjugates
- Labeled and quantitated antibody ready for downstream applications

9. Conclusions

- Antibody labeling as a function of dye concentration for all three isotypes was done in parallel
- Antibody labeling directly from cell media can be done
- AlexaFluor®647 gel scan shows increasing labeling with increase in dye amount
- Commassie gel scan of same gel shows excellent antibody recovery

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