Streamline Your Antibody Enrichment Using Scalable Magnetic Bead-Based Chemistries

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Webinar Outline

• Introduction
• Magnetic Bead-Based Method Overview
• Scalability
• Chemistries
  o Magne™ Protein A and G Beads Applications
    a) Antibody purification
    b) On-bead antibody labeling
  o High Capacity Magne™ Streptavidin Beads Applications
    a) Antibody enrichment
    b) On-bead antibody de-glycosylation
Biological Products

Biologics are complex therapeutic molecules or material from natural sources used to prevent, treat, or cure disease.

- Recombinant factors/cytokines
- Fusion proteins
- Cell-based or tissue therapies
- Antibodies

Purification of Antibodies

Structure of Antibodies

[Diagram showing the structure of an antibody with labeled domains: Antigen binding domain, Fab Section, Sugar chain, and Fc Section binds to Protein G/A.]
Purification of Antibodies (cont.)

Antibody recovery and characterization is required throughout therapeutic antibody drug development

- **Discovery**: Immunogenicity characterization, bioassays, initial biological tests

- **Development & Validation**: Molecular characterization, biophysical analyses, aggregation, solubility, and stability

- **Clinical**: Therapeutic antibody concentration, anti-drug antibody measurement, dose calculation
Purification of Antibodies (cont.)

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- **Clinical**: Therapeutic antibody concentration, anti-drug antibody measurement, dose calculation

**NEED:**
A streamlined workflow for purification of concentrated antibodies for downstream analysis.
Flexible Options for Immunocapture Using Magnetic Beads

- High Capacity Magne™ Streptavidin Beads
- Streptavidin MagneSphere® Paramagnetic Particles
- Magne™ Protein A Beads
- Magne™ Protein G Beads
Antibody Purification: Magnetic vs. Conventional

**Conventional column method**

- Limited throughput
- Expensive instrumentation
- Compatible for large production scale
Antibody Purification: Magnetic vs. Conventional

Conventional column method

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Advantages of magnetic platform

- Convenience: Simple and easy to use
- Throughput: Single sample to high throughput
- Concentrated
- Flexibility: Scalable volumes, manual or automated
Magnetic Bead Handling is Easily Scalable

A platform of various magnetic bead handling capabilities allows flexibility in:

- Sample volume
- Number of samples
- Manual vs. automated

<table>
<thead>
<tr>
<th>Number of samples</th>
<th>1-12</th>
<th>1</th>
<th>1-96</th>
<th>Upto 96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample tubes/plates</td>
<td>0.5ml or 1.5ml tubes</td>
<td>15ml or 50ml tubes</td>
<td>96 well plates</td>
<td>96 well plates/50ml tubes</td>
</tr>
<tr>
<td>Sample volumes</td>
<td>50μl – 1.0ml</td>
<td>1-30ml</td>
<td>50μl-1.0ml</td>
<td>50μl – 50ml</td>
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</table>
Automated Platforms Used With Promega Magnetic Beads

- Magnetic beads are compatible with Maxwell® RSC and HSM platform
- HSM and 96 well MagnaBot® system can be used on robotic platforms (e.g., KingFisher Flex, Hamilton, Tecan, or Biomek) for automation
Flexible Options for Immunocapture Using Magnetic Beads

Target Specific

Isotype, Species, or Tag Specific

Most Antibodies

• High Capacity Magne™ Streptavidin Beads
• Streptavidin MagneSphere® Paramagnetic Particles
• Magne™ Protein A Beads
• Magne™ Protein G Beads
Flexible Options for Immunocapture Using Magnetic Beads

- High Capacity Magne™ Streptavidin Beads
- Streptavidin MagneSphere® Paramagnetic Particles
- Magne™ Protein A Beads
- Magne™ Protein G Beads
Magne™ Protein A&G Beads

Protein A and Protein G are bacterial proteins with high affinity for Fc section of antibodies, and they are commonly used for antibody purification.

- **High Capacity**: Binding capacities up to 25mg per milliliter of settled beads
- **High Purity**: low nonspecific binding
- **Optimized Performance**: small (50μl) to medium (50ml) sample volumes
Magne™ Protein G Beads Performance

Purification from low titer sample

Recovery and scale-up

<table>
<thead>
<tr>
<th>Purification Scale (ml)</th>
<th>Magne™ Protein G Beads (ml)</th>
<th>Mouse antibody purified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG2a (µg)</td>
</tr>
<tr>
<td>1.0</td>
<td>0.05</td>
<td>58</td>
</tr>
<tr>
<td>10.0</td>
<td>0.5</td>
<td>550</td>
</tr>
<tr>
<td>50.0</td>
<td>2.5</td>
<td>2910</td>
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Recoveries are >80% for mlgG2a and ~60% for mlgG1
Magne™ Protein A&G on the Maxwell® RSC

Workflow:

Add Sample

Mix Sample

Capture Magnetic Beads

Bind IgG

Wash Elute

 Therapeutic Antibodies Purified from Cell Culture Media

Rituximab (IgG1) 50μg/ml

Nivolumab (IgG4) 50μg/ml

Panitumumab (IgG2) 50μg/ml

A/G = Protein used for isolation (A-Protein A and G-Protein G); 1/2 = Replicate

Maxwell® RSC (Rapid Sample Concentrator) Instrument: A platform for automated purification of nucleic acid from a range of sample types. Purification relies on sample lysis and binding to paramagnetic particles as the primary separation principle. Up to 16 samples can be prepared simultaneously in 25–60 minutes.
Magne™ Protein A&G in a 96 well format

Magne™ Protein G Beads in a 96-well plate on a MagnaBot® 96 magnet

Magne™ Protein A Beads and Protein G Beads are readily automatable on liquid handlers (e.g., Tecan, Beckman, Hamilton, etc.)

<table>
<thead>
<tr>
<th></th>
<th>Amount of Murine IgG1 Purified (µg)</th>
<th>Amount of Murine IgG2a Purified (µg)</th>
<th>Amount of Murine IgG2b Purified (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magne™ Protein A Beads</td>
<td>2.9</td>
<td>6.4</td>
<td>6.9</td>
</tr>
<tr>
<td>Magne™ Protein G Beads</td>
<td>3.4</td>
<td>7.3</td>
<td>7.1</td>
</tr>
</tbody>
</table>

%CV less than 20%

Antibody purification in a 96-well plate starting from 7.5µg of sample diluted in 150µl of PBS (50µg/ml final concentration). Experiment performed with eight replicates (n = 8).
Application: On-Bead Conjugation:
Magnetic Bead Method for Antibody-Small Molecule Conjugation

Capture antibody with Magne™ Protein A or Protein G Beads.
Wash away contaminants.
Buffer exchange.
Add reactive labeling reagent.
Wash away unreacted labeling reagent.
Elute and neutralize to obtain purified and labeled antibodies.

References
Application: On-Bead Conjugation:
Magnetic Bead Method for Antibody-Small Molecule Conjugation (cont.)

**Advantages:**
- Combined purification and conjugation
- No dialysis steps to separate unconjugated dyes
- No pre-purification or concentration steps necessary

Capture antibody with Magne™ Protein A or Protein G Beads.

Wash away contaminants.

Buffer exchange. Add reactive labeling reagent.

Wash away unreacted labeling reagent.

Elute and neutralize to obtain purified and labeled antibodies.
Application: On-Bead Conjugation:
Magnetic Bead Method for Antibody-Small Molecule Conjugation (cont.)

**Trastuzumab and Control Human IgG Labeling**

Gel images showing labeling of anti-Her2 antibody (Trastuzumab) and control human IgG with Alexa Fluor® 647.

**On-Bead Labeling Does Not Affect the Binding Specificity and Internalization of Antibodies**

SKBR-3 cell surface labeling of Alexa Fluor® 647 trastuzumab (Panel A) and control human IgG Alexa Fluor® 647 (Panel B)—note the punctate staining at 24 hours. Labeling performed at 30nM.
Flexible Options for Immunocapture Using Magnetic Beads

- High Capacity Magne™ Streptavidin Beads
- Streptavidin MagneSphere® Paramagnetic Particles

- Magne™ Protein A Beads
- Magne™ Protein G Beads
High Capacity Magne™ Streptavidin Beads

- Unlike most synthetic molecules that are not present in the serum target, antibodies often need to be detected in the background of similar IgGs.
- Streptavidin conjugated beads allow binding of biotin- antibody or antigen for high specificity enrichment.
High Capacity Magne™ Streptavidin Beads: Performance

- Promega High Capacity Beads: Performance
- Competitor A
- Competitor B
- Competitor C

Capacity of 100µl bead slurry

Biotinylated IgG (µg)
High Capacity Magne™ Streptavidin Beads: Antibody Enrichment

1. Magnetic streptavidin bead
2. Goat anti-Human IgG
3. Biotin
4. Rat Serum + Human ab
5. Cetuximab-Alexa Fluor® 647
6. Elute and neutralize antibody
High Capacity Magne™ Streptavidin Beads: Antibody Enrichment (cont.)

- Magnetic streptavidin bead
  - Goat anti-Human IgG
    - Biotin
  - Rat Serum + Human ab
  - Cetuximab-Alexa Fluor® 647

- Elute and neutralize antibody

- Cetuximab- Anti-EGFR Chimeric mouse/human antibody
- Use of Alexa Fluor® 647 conjugation allows for sensitive detection of antibody recovery
- Use Anti-Human IgG to purify Cetuximab from rat serum
High Capacity Magne™ Streptavidin Bead Performance in 96 Well Automation

Cetuximab-Alexa647 Recovery

Example data from 96 well automation
Pull Down of Antibodies Using Biotinylated Antigen

Workflow for Antigen-Based Antibody Enrichment with High Capacity Magne™ Streptavidin Beads

1. Pre-bind antibodies in sample to biotinylated-antigen
2. Add Magne™ Streptavidin
3. Wash, elute and neutralize
4. Enriched target antibody
Pull Down of Antibodies Using Biotinylated Antigen (cont.)

Recovery of Cetuximab-Alexa647 from rat serum with biotinylated-EGFR

Fluorescent Scan

Coomassie Stain
Application: On-Bead De-Glycosylation
Affinity enrichment and on-bead de-glycosylation

Workflow of affinity enrichment of antibody followed by on-bead de-glycosylation

De-Glycosylation

- Decrease sample complexity prior to analysis
- Determine state of antibody glycosylation
- Characterize glycan structure
Application: On-Bead De-Glycosylation

Affinity enrichment and on-bead de-glycosylation of therapeutic antibodies (cont)

Affinity Enrichment of Spiked Trastuzumab from Human Serum Samples

Affinity Enrichment of Spiked Trastuzumab from Human Serum Samples

On-Bead De-Glycosylation of Captured Trastuzumab

Trastuzumab enriched from the serum sample (Lane 1 & 3); Trastuzumab deglycosylated on-bead using PNGaseF (#F4831) for 2hr (Lane 2) and 4hr (Lane 4)
Webinar Summary

Magnetic beads benefits:

- Scalable process from single sample to high throughput and small to large volume with the same chemistry
- Compatible with many formats, no need for specialized equipment
- Semi-automated, large-volume, high throughput robotics

Magne™ chemistries enable:

- Antibody purification
- On-bead antibody-small molecule conjugation
- Antibody enrichment from serum for PK studies
- Antibody de-glycosylation convenience
Thank You

For more information on materials, contact our Technical Services team:

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