Development of High Capacity Magnetic Beads for Antibody and Protein Purification

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1. Introduction

Recombinant proteins and antibodies are key components in therapeutics, diagnostics, clinical studies and biological research. These applications require access to large numbers of highly purified proteins and antibodies to screen for desirable properties. To address this challenge we developed a magnetic platform as a simple, robust and reproducible method to purify proteins and antibodies from a large panel of samples (1-96) of small to medium volume (50µl-50ml). A key element of the magnetic platform is the use of macroporous, cellulose-based magnetic beads offering high capacity, low non-specific binding, and excellent magnetic response for manual or automated handling. We highlight the advantages of the magnetic platform using multiple examples. We present a **high throughput purification of antibodies from variety of samples including cell-media, ascites fluid and serum** using high capacity magnetic Protein A and Protein G beads – a method that is **readily automatable on robotic liquid handlers including Tecan and Beckman.** We describe a **novel on-bead antibody-small molecule conjugation method** that combines antibody labeling and purification in a single workflow precluding the need for antibody pre-purification, concentration, or dialysis prior to labeling. Lastly we present a **high-throughput protein purification example for purifying multiple HIS-tagged proteins** expressed in *E. coli* and purified with high capacity Magnetic HIS affinity beads.

2. Magnetic Bead Platform

Manual mode

Number of Samples	1–12	1	1–96
Processing Tubes/Plates	0.5ml or 1.5ml microcentrifuge tubes	15ml or 50ml polypropylene tubes	96-well standard or deep-well plates
Input Sample Volumes	50µl–1.0ml	1–50ml	20µl–1.0ml

Automated mode

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

- **Advantages of magnetic platform**
- Convenience: Simple and easy to use
- Throughput: 1-96 samples can be processed in parallel
- Sample volumes of 20µl to 50ml can be easily handled
- Sample concentration from dilute samples
- Flexibility: Manual or automated

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

Oriented Attachment of Protein A and Protein G

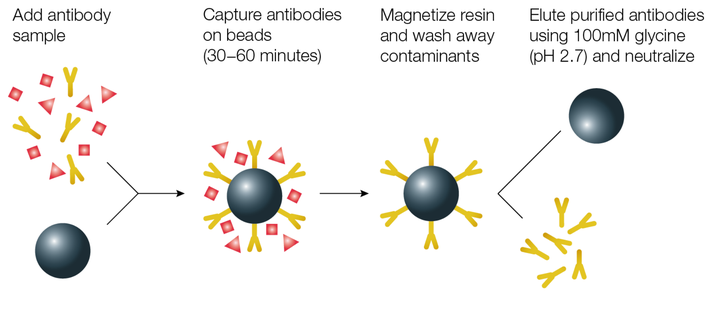
- Macroporous cellulose bead, 30-80µm size
- Low non-specific binding for high purity antibody purification
- Covalent and oriented immobilization of Protein A and Protein G using HaloTag® technology
- High antibody binding capacity of ~25mg/ml
- Efficient binding of antibody from various species and isotypes (Table)

Species	Isotype	Magne™ Protein G Beads	Magne™ Protein A Beads
Bovine	IgG	33	36.2
Goat	IgG	26	26.2
Human	IgA	0.4	4.8
	IgG1	39.4	35.2
	IgG2	32.6	34.4
	IgG3	37.6	0
	IgG4	38	28.8
	IgM	0	8.2
Mouse	IgG1	30.4	18.6
	IgG2a	31.6	33.2
	IgG2b	31.4	29.6
	IgG3	9.2	16
Porcine	IgG	27.6	35.2
Rat	IgG1	34.2	29.4
	IgG2a	33	0
	IgG2b	31.2	0
Sheep	IgG	25.6	26.6

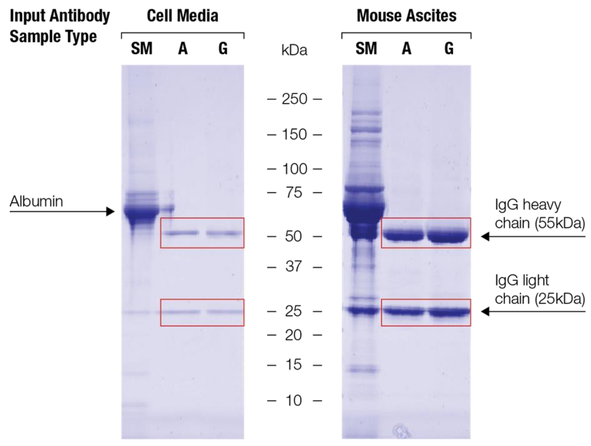
50µg antibody in 1ml purified with 50µl bead slurry in triplicate (CV ≤10%)

4. Magne™ Protein A and G Beads: Simple Protocol, High Capacity, Scalability

Simple Protocol



High Purity



SM = Starting Material
A = Antibody purified with Magne™ Protein A Beads
G = Antibody purified with Magne™ Protein G Beads

Scalable Purification

Purification Volume (ml)	Input Starting Material (ml)	Buffer; 10X PBS (ml)	Added Magne™ Protein G Beads (ml)	Yield of Purified IgG2A (µg)	Yield of Purified IgG1 (µg)
1.0	0.9	0.1	0.05	**58**	**25**
10.0	9.0	1.0	0.5	**550**	**230**
50.0	45.0	5.0	2.5	**2910**	**980**

Antibody purification yield from cell media for two mouse isotypes (IgG2A and IgG1) expressed in cell media. Starting antibody concentration: 50–60µg/ml.

Parallel Sample Processing in 96-well plate

	Amount of Murine IgG1 Purified (µg)	Amount of Murine IgG2a Purified (µg)	Amount of Murine IgG2b Purified (µg)
Magne™ Protein A Beads	2.9	6.4	6.9
Magne™ Protein G Beads	3.4	7.3	7.1

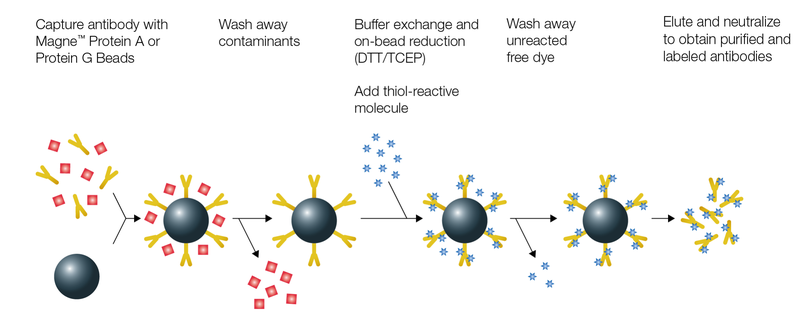
CV<20%

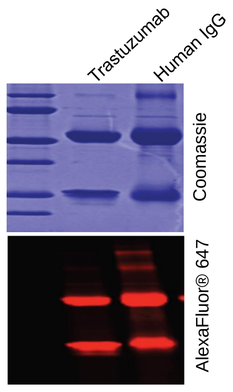
Antibody purification in a 96-well plate starting from 7.5µg of sample diluted in 150µl of PBS (50µg/ml final concentration). Experiment performed with eight replicates (n = 8).

- Beads are readily used on automated liquid handlers including Tecan, Beckman, and Hamilton platforms

5. On-Bead Antibody Conjugation

Combined Purification and Conjugation of Antibody from Cell Media

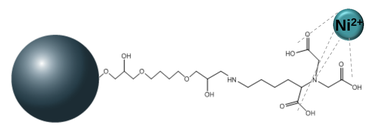




- Compatible with amine and thiol labeling reagents
- Automatable for 1-96 samples at a time
- Conjugate (label) at 50µl - 50ml quantities
- Method that bypasses the pre-purification, dialysis, and concentration steps that are required for in-solution labeling

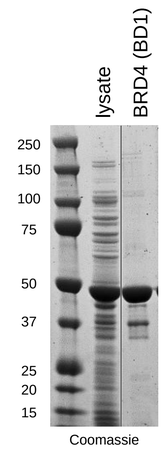
6. HIS Tag Protein Purification using Magnetic HIS Beads

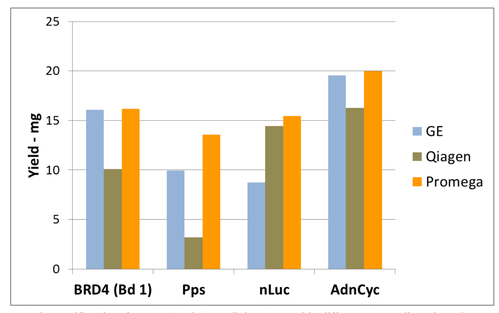
High Capacity Magnetic HIS Beads (under development)



- Macroporous cellulose bead, 30-80µm size
- Tetradentate Ni^{2+} chelate
- Capacity: >20mg/ml of settled beads
- Compatible with buffers containing EDTA (<0.1mM), DTT (<10mM)

7. Purification of *E. coli* Expressed Proteins using High Capacity Magnetic HIS Beads





Protein purification from 125 µl *E. coli* lysates with different suppliers beads according to manufacturer's directions. Yield normalized to 1 ml of settled beads

Scalable Purification

Purification Volume (ml)	Starting *E. coli* culture Material (ml)	10X FastBreak™ Lysis Reagent (ml)	DNase1 (µl)	Wash buffer (ml)	Elution volume (ml)	Yield of Purified protein (mg)
1.0	0.9	0.1	1	0.5	0.1	**0.2**
10.0	9.0	1.0	10	5	1	**2**
50.0	45.0	5.0	50	25	5	**11.8**

Purification of HIS-tagged human BRD4 (BD1) (bromodomain1) protein from different volumes of *E.coli* lysates

Parallel Purification in 96-well and 24-well plates

	Tag placement	96-well plate yield (µg)	%CV	24-well plate yield (µg)	%CV
BRD4 (BD1)	N-	**99**	1.2	**416**	8.2
Plant diaphorase	C-	**30**	1.9	**136**	4.2
GFP	N-	**161**	12.2	**674**	2.9
Phosphoenolpyruvate kinase	N-	**94**	10.9	**332**	1.7
UMP-CMP kinase	N-	**103**	2.4	**242**	12.9

Purification of HIS-tagged proteins in 96-well and 24-well plates using 50 µl and 200µl of magnetic HIS beads slurry

- Better performance than competing products from GE and Qiagen
- Easy-to-handle beads that minimize sample losses
- Flexible sample volume inputs from 20µl to 50ml

8. Conclusions

- Magnetic bead platform enables parallel sample processing for protein purification
- High capacity magnetic beads provide exceptional yields of antibodies and HIS-tagged proteins that rival those of non magnetic resins
- Magne™ Protein A and Magne™ Protein G beads allow both antibody purification and on-bead antibody conjugation in both manual and automated formats