EXTRACTMAN®: Protein G Magnetic Bead Comparisons for Immunoprecipitation

APPLICATION NOTE AN1015

APPLICATION BENEFITS

Magnetic beads vary in size, shape, uniformity, weight, core composition, coating, magnetic strength, hydrophobicity, and conjugation chemistries. The physical properties of the beads determine their performance on EXTRACTMAN®. Superparamagnetic Protein G particles from three suppliers were tested with a simple immunoprecipitation protocol to evaluate their performance on EXTRACTMAN.

SOLUTIONS

EXTRACTMAN®'s exclusion-based sample preparation (ESP™) technology is compatible with the physical properties of Dynabeads®, SureBeads™, and Pierce™ Magnetic Beads. The availability of a variety of NEB magnetic beads, covalently immobilized affinity ligands, including Protein A, Protein A/G, and streptavidin, coupled with the flexibility of EXTRACTMAN, makes it easy to adapt existing protocols and even develop completely new magnetic bead applications.

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INTRODUCTION

Immunoprecipitation (IP) and Co-IP are common techniques for isolating proteins and identifying protein-protein interactions, respectively. Exclusion-based sample preparation (ESP™) with EXTRACTMAN® (Figure 1) allows for rapid, yet gentle washing of protein solutions during these procedures. EXTRACTMAN has been shown to be ideal for isolating elusive, weakly-bound protein complexes by Co-IP using Dynabeads® and for immunoprecipitation of RNA binding proteins.[1,2] This application note describes a comparison of superparamagnetic particles (magnetic beads) conjugated to Protein G for immunoprecipitation using EXTRACTMAN.

Figure 1
EXTRACTMAN®

The conventional tube-based immunoprecipitation protocol was first compared to the EXTRACTMAN method using Dynabeads®. The comparison demonstrated that ESP™ is superior to conventional IP methods as demonstrated by Western blotting and silver staining. Protein G-conjugated Dynabeads® (Thermo Fisher), SureBeads™ (Bio-Rad) and Pierce™ Magnetic Beads were evaluated for use with EXTRACTMAN and were compatible for use with EXTRACTMAN for immunoprecipitation. NEB, Miltenyi, and agarose beads were also evaluated based only on their physical properties. All beads, except for the very small Miltenyi beads, were determined suitable for use on EXTRACTMAN.

MATERIALS AND METHODS

Pre-Immobilization of Antibody to Magnetic Protein G Beads

Dynabeads®, Protein G for Immunoprecipitation (Invitrogen), SureBeads™ Protein G Magnetic Beads (Bio-Rad) and Pierce™ Protein G Magnetic Beads were pre-bound to mAb 8RB13 by washing 20 µL of beads four times with 200 µL IP Buffer (Tris-Buffered Saline + 0.01% Triton X-100) using a magnetic rack and then incubated with 50 µg/mL mAb 8RB13 in IP buffer at room temperature for 20 minutes with gentle mixing every five minutes. mAb 8RB13 is a polyol-responsive antibody that binds the 8RB13 epitope present in many bacterial RNA polymerases.[3]

GILSON®

Antigen Binding to Beads

Antibody-bound beads were washed three times with IP buffer using a magnetic rack before addition of 300 µL *E. coli* whole cell lysate containing 8RB13-epitope-tagged enhanced green fluorescent protein (8RB13-etEGFP) that was prepared as previously described.[4] Samples were incubated on ice for 30 minutes and mixed by tapping every five minutes. A lysate sample was retained for Western blotting and silver staining.

Conventional Wash and Elution

A magnetic rack was used to wash the beads four times with 100 µL of IP Buffer. The beads were resuspended with gentle tapping between each one minute wash. The beads were transferred to a clean tube in the final wash buffer to reduce background. Samples from each step were retained for analysis by Western blotting and silver staining.

EXTRACTMAN® Wash and Elution

The EXTRACTMAN plate was loaded with 250 µL of IP reaction (lysate and beads). IP buffer was loaded into the 110 µL wash wells. EXTRACTMAN was used to transfer the beads through each well with a hold time of five to six seconds for the beads to collect on the bead strip. The beads were collected after the final wash and eluted by heating to 75˚C for five minutes in SDS-PAGE sample buffer. Samples were retained from each well for analysis by Western blotting and silver staining.

SDS-PAGE, Western blot, and Silver Staining

Samples heated as above in SDS-PAGE sample buffer were run on a 4%–12% NuPAGE gradient gel (Invitrogen). Identical gels were run for parallel Western blot and silver staining analyses. Western blots were probed with mAb1GFP52 for detection of 8RB13-etEGFP.

RESULTS AND DISCUSSION

Conventional Immunoprecipitation versus EXTRACTMAN® Method

The conventional IP method was compared with the EXTRACTMAN protocol. 8RB13-etGFP was immunoprecipitated with Protein G Dynabeads® that were preloaded with mAb (8RB13). Alternatively, one could form the immune complexes in solution if the antibody has weak affinity, slow binding kinetics, or the target antigen is of low abundance. Western blot analysis with anti-GFP antibody was used to analyze "load" (lysate) and "unbound" (lysate after removal of beads). Analysis of the washes demonstrates that the longer washes of the conventional methods result in some loss of antigen that does not occur when washing with EXTRACTMAN (Figure 2A). Silver staining demonstrates that the rapid washes of the EXTRACTMAN protocol did not elevate background (Figure 2B). Endogenous RNA polymerase contains the 8RB13 epitope and is therefore detected by silver staining as it was also immunoprecipitated with the anti-RNA polymerase mAb (8RB13).

Bead Evaluation

Magnetic beads were evaluated for suitability on EXTRACTMAN® by visual observation. Beads that both adhered to the bead capture strip in less than 20 seconds and dispersed easily into the well were given a "pass" rating. The results are summarized in Table 1. Dynabeads®, SureBeads™, Pierce™ Magnetic Beads, and NEB® Magnetic Beads met the pass criteria. Miltenyi MicroBeads are very small (0.05 µm) and have insufficient magnetic force to collect onto the bead capture strip. Agarose beads and other porous matrices require longer exposures to wash buffers to facilitate diffusion of contaminants from the bead matrix. Thus, agarose beads can be used but will not fully exploit the speed advantage offered by EXTRACTMAN.

Table 1

Magnetic bead compatibility with EXTRACTMAN®

Core Bead	Cat Number (Protein G)	Size (µM)	Compatible with EXTRACTMAN
Dynabeads™	10003D	2.8	Yes
Bio-Rad SureBeads™	161-4021	2.4 to 3.4	Yes
Pierce™ Magnetic Beads	88802	1	Yes
NEB Magnetic Beads	S1430S	2	Yes
µMACS™ Miltenyi MicroBeads	130-071-101	0.05	No
Magnetic Agarose Beads	N/A	Various	Longer washes required

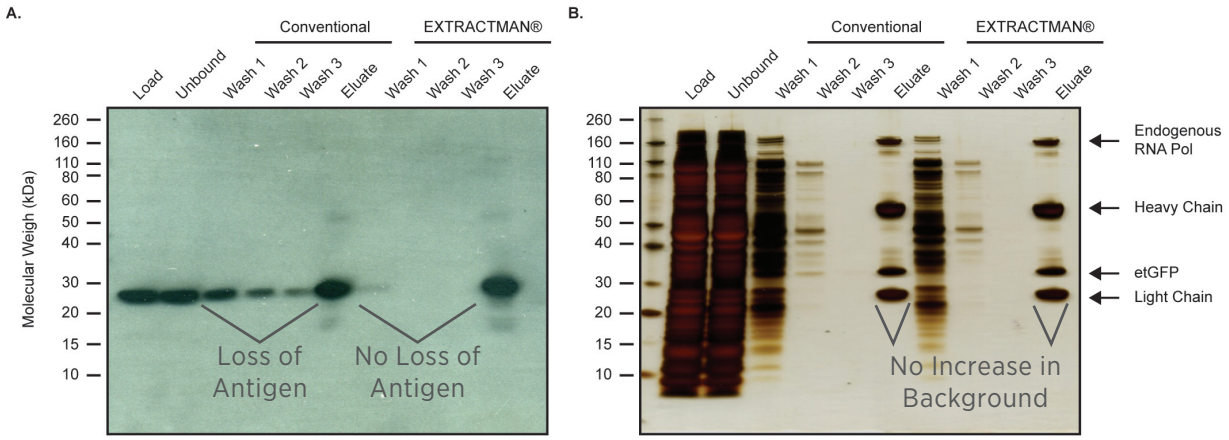

Figure 2

EXTRACTMAN® Method Outperforms Conventional Immunoprecipitation

A conventional IP protocol was compared to the EXTRACTMAN® method using Dynabeads®. Load (lysate), unbound (lysate after removal of beads), washes, and elution were analyzed by Western blot analysis with mAb 1GFP52 for the detection of epitope-tagged GFP (etGFP) (A) and silver staining (B). Note the substantial loss of 8RB13-etGFP in the washes of the conventional IP.

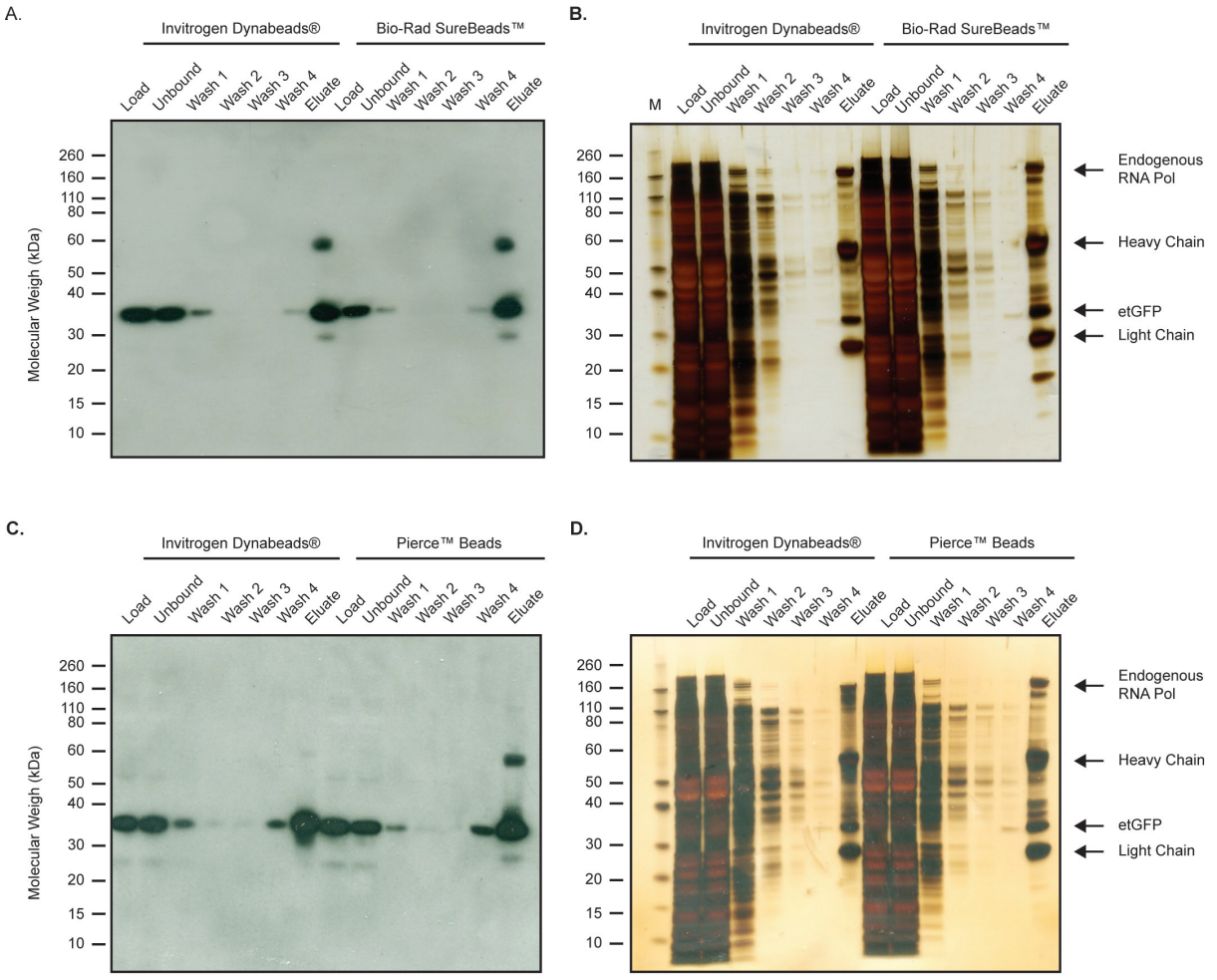

Figure 3

EXTRACTMAN® Technology Is Compatible with Protein G-Conjugated Dynabeads®, SureBeads™, and Pierce™ Magnetic Beads

Load (lysate), unbound (lysate after removal of beads), washes, and elution were analyzed by Western blot analysis with mAb 1GFP52 for the detection of epitope-tagged GFP (etGFP) (A, C) and silver staining (B, D) to compare Dynabeads® to SureBeads™ and Pierce™ Magnetic Beads.

Compatibility of Dynabeads, SureBeads, and Pierce Magnetic Beads with EXTRACTMAN®

IPs were performed with EXTRACTMAN using Dynabeads®, SureBeads™, and Pierce™ Magnetic Beads conjugated to Protein G. All analyses were performed in the same manner as the previous experiment. Western blot analysis detected little difference in the immuprecipitation of 8RB13-etGFP with SureBeads™ (Figure 3A) and silver staining did not detect any significant difference in background between Dynabeads® and SureBeads™ (Figure 3B). Similarly, Western blot analysis detected little difference in the immunoprecipitation of 8RB13-etGFP with Pierce™ Magnetic Beads (Figure 3C) and silver staining did not detect any significant difference in background between Dynabeads® and Pierce™ Magnetic Beads (Figure 3D).

The findings of this study can be translated to IP, Co-IP, RNA immunoprecipitation (RIP), and antibody purification protocols. Protein A-conjugated magnetic beads are available from each of the suppliers with analogous cores and surface chemistries and could be substituted for applications requiring Protein A in place of Protein G. Further, analogous beads are available with streptavidin (for isolation of biotinylated antibodies and nucleic acids), Ni-NTA (for affinity purification of His-tagged proteins) and NHS-activated surfaces (for amine-based conjugation of any protein) providing many opportunities to apply ESP™ technology to common methods and to create new applications using EXTRACTMAN.

CONCLUSIONS AND BENEFITS

- EXTRACTMAN® out-performs conventional immunoprecipitation in yield.
- Dynabeads® Protein G for Immunoprecipitation (Invitrogen), SureBeads™ Protein G Magnetic Beads (Bio-Rad) and Pierce™ Protein G Magnetic Beads were comparable.
- Antigen is retained better with EXTRACTMAN versus conventional IP without increasing background signal.
- Any beads not listed in this application note should be pre-validated by visually evaluating performance on EXTRACTMAN.
- Conventional immunoprecipitation protocols are easily converted to EXTRACTMAN-compatible methods.

REFERENCES

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4. E. S. Stalder, L. H. Nagy, P. Batalla, T.M. Arthur, N. E. Thompson, and R. R. Burgess, The epitope for the polyol-responsive monoclonal antibody 8RB13 is in the flap-domain of the beta-subunit of bacterial RNA polymerase and can be used as an epitope tag for immunoaffinity chromatography. *Protein Expr. Purif.* 77(1), 26-33 (2011).

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ORDERING INFORMATION

Description	PN	QTY
EXTRACTMAN® Starter Kit • EXTRACTMAN® Handle Assembly • EXTRACTMAN® Base Assembly • Bead Collection Strips (qty. 5) • 15 µL Extraction Plates (qty. 5) • User's Guide	22100000	1
EXTRACTMAN® Bead Capture Strips	22100007	25
EXTRACTMAN® 15 µL Extraction Plates	22100008	25