

The Use of PureProteome™ Magnetic Beads for Faster Immunoprecipitation

ABSTRACT

Traditional immunoprecipitation using protein A or protein G agarose beads has long been a popular research method. Recently, scientists have replaced the agarose beads with magnetic beads for easier retrieval of the immunoprecipitated sample. Millipore's new PureProteome magnetic beads offer easy sample retrieval and the added benefit of faster immunoprecipitation. Side-by-side comparisons of agarose and magnetic beads in an immunoprecipitation assay demonstrated that the use of PureProteome beads yielded equivalent results while reducing incubation time from two hours to ten minutes.

INTRODUCTION

Both protein A, from *Staphylococcus aureus*, and protein G, which is produced by various *Streptococcus* species, can be extremely useful research tools for applications involving antibodies, as the Fc portion of a variety of immunoglobulins is known to bind to them. Most immunoprecipitation protocols take advantage of this and use protein A or protein G beads to capture the antibody-labeled protein of interest.

While traditional agarose bead methods require minutes of centrifugation to pellet samples, the PureProteome system uses magnetism to gently isolate proteins in seconds. To bind the immunoglobulins, samples are incubated with PureProteome protein A or protein G magnetic beads for a short period of time. Unlike the larger, more viscous agarose beads, PureProteome beads offer increased reaction kinetics that reduce incubation times from hours to mere minutes with equivalent results, as demonstrated in Figure 1. The protein of interest is then isolated using the magnetic Magna GriP™ rack, which gently immobilizes the highly-visible beads against the side of the tube. This allows quick and easy aspiration and eliminates the need for centrifugation, greatly reducing bead loss and thereby improving reproduc-

ibility. Several wash steps follow to remove unbound proteins. Finally, the bound proteins are eluted at high purity. This magnetic system is a convenient format for serum depletion, immunoprecipitation, or other applications that employ protein A or protein G.

RESULTS & DISCUSSION

As shown in Figure 1, both magnetic beads and agarose beads gave very similar results in parallel immunoprecipitation assays. Both offered equivalent yields of p53 protein and low non-specific binding. Using the same reagents and lysates, the PureProteome magnetic beads yielded similar results to agarose beads in a significant time reduction with no detectable ligand leaching. The immunoprecipitation using the PureProteome magnetic beads required only 10 minutes to provide results equivalent to those that the agarose immunoprecipitation achieved in two hours.

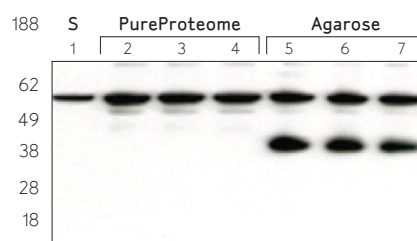
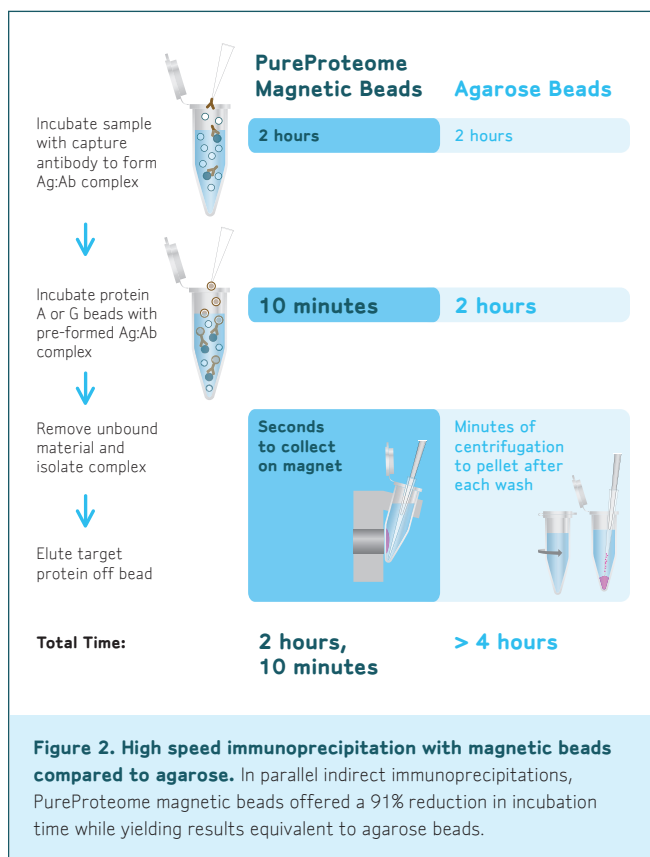


Figure 1. Immunoprecipitation of p53 from A431 lysates using magnetic and agarose beads. Western blot detection of parallel agarose and magnetic immunoprecipitations of p53, showing input material (lane 1), immunoprecipitation with PureProteome protein G magnetic beads (lanes 2-4) or protein G agarose beads (lanes 5-7).

METHODS

Immunoprecipitation

Whole-cell lysates (300 µg of protein) prepared from human epidermoid carcinoma (A431) cells were incubated with 5 µg of a mouse monoclonal antibody specific for the tumor suppressor protein/transcription factor p53 (Catalogue No. 05-224). PureProteome protein G magnetic beads or protein G agarose beads were then added to the reaction to capture the immune complexes following the indirect immunoprecipitation protocol. The pre-formed immune complex was incubated with magnetic beads at room temperature for 10 minutes or agarose beads at 4 °C for 2 hours.



Western Blot Detection

Samples of bound fractions were separated by SDS-PAGE prior to semi-dry transfer to an Immobilon®-P blotting membrane. Immunodetection was performed with the SNAP i.d.™ protein detection system using a rabbit polyclonal primary antibody specific for p53 (Catalogue No. AB565, diluted 1:1,000) and a horseradish peroxidase conjugated goat anti-rabbit secondary antibody (Catalogue No. AP132P, diluted 1:40,000). The blot shown was visualized using the Immobilon Western HRP substrate and represents a 1-minute exposure to x-ray film.

ORDERING INFORMATION

Kits & Assays

Description	Qty/Pk	Catalogue No.
PureProteome Protein A Magnetic Beads	10 mL	LSKMAGA10
	2 x 1 mL	LSKMAGA02
PureProteome Protein G Magnetic Beads	10 mL	LSKMAGG10
	2 x 1 mL	LSKMAGG02
Pure Proteome Nickel Magnetic Beads	10 mL	LSKMAGH10
	2 x 1 mL	LSKMAGH02
Magna GriP Rack		20-400

TO PLACE AN ORDER OR RECEIVE TECHNICAL ASSISTANCE

In the U.S. and Canada, call toll-free 1-800-MILLIPORE (1-800-645-5476)

Outside of North America, please visit www.millipore.com/offices

For technical service, please visit www.millipore.com/techservice



www.millipore.com

ADVANCING LIFE SCIENCE TOGETHER™
Research. Development. Production.

Millipore, Upstate, Chemicon, and Immobilon are registered trademarks of Millipore Corporation. The M mark, Advancing Life Science Together, PureProteome, SNAP i.d., and Magna GriP are trademarks of Millipore Corporation.
Lit. No. AN2394EN00 Printed in U.S.A. 07/09 BS-GEN-09-02130
© 2009 Millipore Corporation, Billerica, MA 01821 U.S.A. All rights reserved.