Immunoprecipitation Performance of SureBeads™ Protein A Magnetic Beads vs. Protein A Agarose Beads

Galina Fihman,[1] Aman Tyagi,[2] Sara Heitkamp,[2] and Dalia Shezifi[1]
[1]Bio-Rad Laboratories, Gutwirth Park, Technion, Haifa 32000, Israel
[2]Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA, USA

Electrophoresis and Blotting

Bulletin 6594

Introduction

In a laboratory study, we compared the immunoprecipitation (IP) performance of new SureBeads Magnetic Beads with that of agarose beads.

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a low-abundance flavoenzyme that utilizes a reduced pyridine nucleotide cofactor NADH or NADPH to catalyze the direct two-electron reduction of a broad range of quinones, which prevents the production of free radical species. Many tumors express high levels of NQO1 (Ross et al. 2000, Siegel et al. 2012) and altered expression of the protein is associated with Alzheimer's disease (SantaCruz et al. 2004, Wang et al. 2006). In addition, NQO1 has been shown to play an important role in the bioactivation of several quinone-containing antitumor drugs (Siegel et al. 2012). Here, NQO1 was immunoprecipitated from HeLa cell lysate using either Bio-Rad's SureBeads Protein A Magnetic Beads or protein A agarose beads from another company and its presence confirmed via western blotting.

Methods

In this IP experiment, 0.5 ml HeLa lysate diluted to 4 mg/ml in RIPA buffer was used as the antigen sample. Identical volumes (50 µl) of either SureBeads Protein A Magnetic Beads (cat. #161-4013) or protein A agarose beads were used to immunoprecipitate NQO1 from the sample. The concentration of the IP antibody was varied (0.5, 2, 4, or 8 µg per IP reaction) to evaluate the performance of each bead type as a function of the amount of antibody.

The magnetic and agarose beads were incubated with rabbit-anti NQO1 antibody (ab34173, Abcam), followed by incubation with the diluted HeLa lysate.

After each incubation step, the beads were thoroughly washed with PBS + 0.1% Tween 20 buffer. The magnetic beads were washed using the 16-tube SureBeads Magnetic Rack (cat. #161-4916) while the agarose beads were washed by centrifugation. The eluted protein sample was run on an Any kD™ Criterion™ TGX Stain-Free™ Gel (cat. #567-8124) and transferred using the Trans-Blot® Turbo™ Transfer System (cat. #170-4155) and midi PVDF transfer packs (cat. #170-4157).

Western blotting was performed using 1 µg/ml goat anti-NQO1 primary antibody (ab2346, Abcam) and 1:10,000 anti-goat HRP secondary antibody (805-035-180, Jackson ImmunoResearch). The bands were detected using Clarity™ Western ECL Substrate (cat. #170-5061) and the blot was imaged using the ChemiDoc™ MP System (cat. #170-8280).

Results

Figure 1 shows the western blotting results for the IP experiment performed using either SureBeads Magnetic Beads or agarose beads. The signal intensity with the magnetic beads is much brighter than with the agarose beads — even when 4x more antibody is used in the agarose experiment (compare lane 2, SureBeads Magnetic Beads with 2 µg antibody, to lane 9, agarose beads with 8 µg antibody).

The SureBeads Magnetic Beads protocol is faster and easier than the traditional agarose protocol because the beads magnetize to the side wall of the tube, not the bottom, increasing the ease of pipetting during the wash steps and minimizing sample loss.

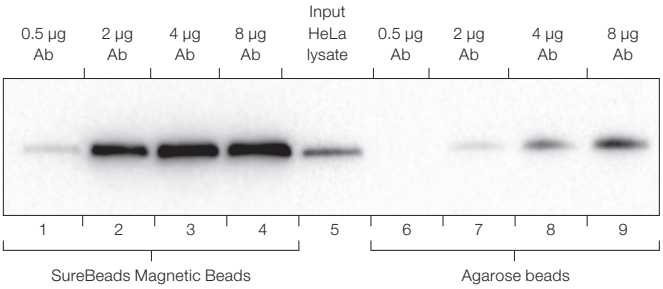

Fig. 1. Comparison of SureBeads Magnetic Beads and agarose beads. SureBeads Magnetic Beads were washed using the 16-tube magnetic rack while the agarose beads were washed by centrifugation.

References

Ross D et al. (2000). NAD(P)H:quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. Chem Biol Interact 129, 77–97.

SantaCruz KS et al. (2004). Regional NAD(P)H:quinone oxidoreductase activity in Alzheimer's disease. Neurobiol Aging 25, 63–69.

Siegel D et al. (2012). NAD(P)H:quinone oxidoreductase 1 (NQO1) in the sensitivity and resistance to antitumor quinones. Biochem Pharmacol 83, 1033–1040.

Wang B et al. (2006). Association analysis of NAD(P)H:quinone oxidoreductase gene 609 C/T polymorphism with Alzheimer's disease. Neurosci Lett 409, 179–181.