Application Note

Magna ChIP™ protein A/G bead blends simplify the ChIP process and reduce background signals

Introduction

Chromatin immunoprecipitation (ChIP) has been widely adapted for the study of gene-specific and genome-wide distribution of specific DNA- and RNA-binding proteins or protein modifications. Similar to standard protein immunoprecipitation assays, ChIP involves isolation of immunocomplexes using a solid medium, such as agarose or magnetic beads, coupled to either IgG-binding recombinant protein A or protein G. In a typical ChIP experiment either protein A or G is selected for enrichment depending on the antibody isotype. However, proteins A and G possess differing affinities for human and mouse IgGs (Table 1). Complicating this choice, for some antibody isotypes there is affinity for both protein A and G. In addition, we have observed that independent of the isotype the affinity of a specific antibody for protein A or G can vary depending on the specific clone, purification method, and source.

During the development of ChIP-validated antibody/primer sets (ChIPAb+™), we screen multiple candidate antibodies to determine which antibodies perform best in ChIP. Because these antibodies are from a range of species and include both monoclonal and polyclonal antibodies, preference for protein A or G typically varies. To reduce the impact of bead type as a variable and remove the need to use different types of beads for screening these antibodies, we began evaluating ChIP performance using blends of Magna ChIP™ protein A and protein G beads.

Species	Immunoglobulin	Binding to Protein A	Binding to Protein G
Human	IgG (normal)	++++	++++
	IgG_1	++++	++++
	IgG^2	++++	++++
	IgG_3	–	++++
	IgG_4	++++	++++
	IgM	–	–
	IgA_1	–	–
	IgA_2	+	-
	IgD	-	-
	IgE	–	–
Mouse	IgG_1	+	++++
	IgG_{2a}	++++	++++
	IgG_{2b}	+++	+++
	IgG_3	++	+++
	IgM	+	-
	IgA	-	-
	IgE	-	-
Rat	IgG_1	–	+
	IgG_{2a}	–	++++
	IgG_{2b}	–	++
	IgG_{2c}	+	++
Goat	IgG	+/-	++
Guinea Pig	IgG_1	++++	Not Tested
	IgG_2	++++	Not Tested
	IgM	-	Not Tested
Bovine	IgG_1	+	+++
	IgG_2	+++	+++
	IgA	-	-
Rabbit	IgG	++++	+++
Sheep	IgG_1	-	+++
	IgG_2	+++	+++

Table 1. Binding affinities for protein A and protein G to various immunoglobulin isotypes[1-8]

Experiments comparing protein A vs. protein G vs. protein A/G magnetic bead blends revealed that a mixture of protein A and G beads worked well with a wide variety of antibody isotypes. The use of Protein A/G bead blends eliminated the need to consider which beads or kit to use in order match a particular antibody/bead binding affinity combination. In addition to simplifying the procedure, we found that compared to the use of either protein A or protein G alone, protein A/G magnetic bead blends improved signal-to-noise ratios without decreasing the recovery of input chromatin. Here, we describe the use of magnetic protein A/G bead blends and present data demonstrating improved signal-to-noise ratios though the use of Magna ChIP™ protein A/G kits and beads.

Methods

Chromatin from HeLa cells (1 x 10^6 cell equivalents per IP) were subjected to chromatin immunoprecipitation using ChIPAb+™ kits as a source of antibody, normal IgG and positive control primer set (Cat. No. 17-601 and Cat. No.17-646) as well as materials provided in the EZ-Magna ChIP™ A kit (Cat. No. 17-408), EZ-Magna ChIP™ G kit (Cat. No. 17-409) or the EZ-Magna ChIP A/G kit (Cat. No. 17-10086). Successful IP of protein-associated DNA fragments was verified by qPCR using ChIP primers indicated. Please see respective ChIPAb+™ kit protocol for details of experimental procedure.

Results and Discussion

In evaluations of multiple antibodies, Magna ChIP™ A/G magnetic beads were as effective as protein A or G magnetic beads alone in isolating DNA-associated immunocomplexes. Regardless of the antibody used Magna ChIP protein A/G bead blends significantly reduced background signals in control IgG samples (Figures 1 and 2).

In addition, the use of an A/G blend has no impact on the amount of material recovered as compared to the use of a single bead type. As shown in figures 1A and 2A there is no significant difference in mean Cq for specific

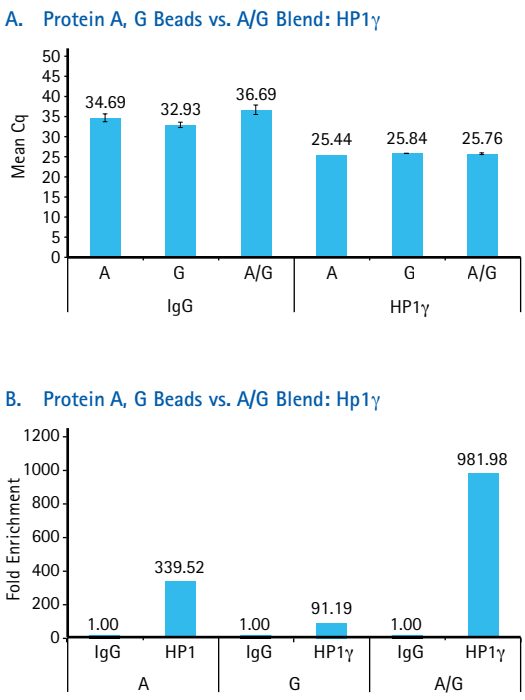

Figure 1.

qPCR analysis of DNA immunoprecipitated with protein G-purified mouse monoclonal anti-Hp1γ or IgG and isolated with protein A, G, and A/G blend magnetic beads. Hp1γ- and IgG-associated DNA were immunoprecipitated from HeLa cells as described in the user manual for the ChIPAb+™ 17-646 kit. Immunocomplexes were collected using 20 µL protein A, protein G, or A/G blend magnetic beads and a magnetic stand. Data are shown as Mean Cq with standard deviation of quadruplicate PCR (A) and fold enrichment (B).

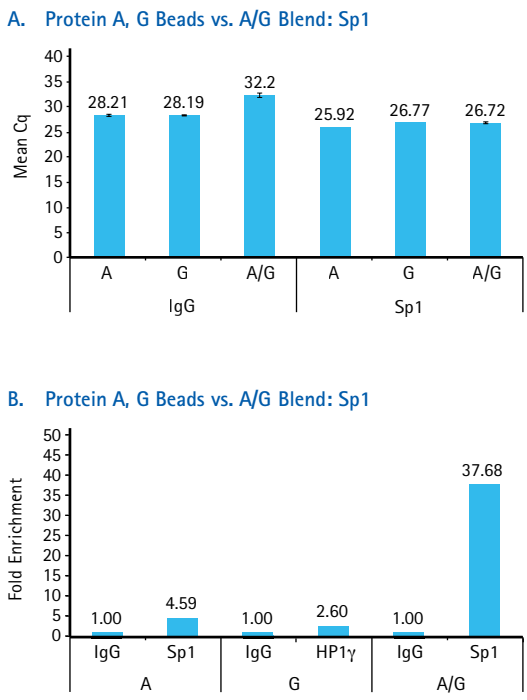

Figure 2.

qPCR analysis of DNA immunoprecipitated with protein A-purified rabbit polyclonal anti-Sp1 or IgG and isolated with protein A, G, and A/G blend magnetic beads. Sp1- and IgG-associated DNA were immunoprecipitated from HeLa cells as described in the user manual for the ChIPAb+™ 17-601 kit. Immunocomplexes were collected using 20 µL protein A, G, or A/G blend magnetic beads and a magnetic stand. Data are shown as Mean Cq with standard deviation of quadruplicate PCR (A) and fold enrichment (B).

antibody samples (HP1γ and Sp1) when using magnetic protein A/G blends for enrichment. In contrast, in Figures 1A and 2A the matched IgG negative control for the protein A/G blends shows a significantly later mean Cq (indicating less background noise) as compared to reactions using protein A or G alone.

Because protein A/G blends reduce the background while allowing for similar recoveries to a single bead reaction, much higher fold enrichment is observed (Figures 1B and 2B). We have observed similar results for virtually all antibodies tested to date and in no cases observed a negative impact on either background signal or recovery of input when using protein A/G blends.

Conclusions

The data presented here demonstrate that the use of magnetic protein A/G blend beads results in higher fold enrichment in ChIP experiments, compared to protein A or G alone. While the recovery of input appears to be unaffected, the sharp decrease in background signal suggests that the use of magnetic A/G blends can allow for greatly improved signal to noise ratios.

We have tested multiple antibodies using A/G blends and found that, while the magnitude of this improvement may vary from antibody to antibody, in almost all cases, an increase in fold enrichment is observed. For the very few antibodies where we did not see an improvement, the impact of using a protein A/G blend was neutral. Our data suggests that protein A/G blends will typically improve fold enrichment and allow better detection of less abundant chromatin complexes by qPCR.

While reliable ChIP can be performed with protein A or protein G beads alone, we find that by using magnetic protein A/G blend beads, ChIP assays can be reliably performed using a wider variety of antibody isotypes. In addition to allowing for lower backgrounds and robust signals, this approach enables laboratories to implement a unified approach for ChIP and as well as eliminate the additional cost of purchasing multiple kits or vials of magnetic beads containing only a single bead type.

Ordering Information

Protein A/G Kits and Beads

Description	Cat. No.
Magna ChIP™ A/G Chromatin Immunoprecipitation Kit	17-10085
EZ-Magna ChIP™ A/G Chromatin Immunoprecipitation Kit	17-10086
Magna ChIP™ HiSens Chromatin Immunoprecipitation Kit	17-10460
EZ-Magna ChIP™ HiSens Chromatin Immunoprecipitation Kit	17-10461
Magna ChIP-Seq™ Chromatin Immunoprecipitation and Next Generation Sequencing Library Preparation Kit	17-1010
Magna ChIP™ HT96 Chromatin Immunoprecipitation Kit	17-10077
EZ-Magna ChIP™ HT96 Chromatin Immunoprecipitation Kit	17-10078
Magna ChIP™ Protein A+G Magnetic Beads	16-663

Related Products

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Description	Cat. No.
ChIP Ab+™ Validated Antibody/Primer Sets	See website
PureEpi™ Chromatin Preparation and Optimization Kit	17-10082
Magna ChIP™ Protein A Magnetic Beads	16-661
Magna ChIP™ Protein G Magnetic Beads	16-662
PureProteome™ Magnetic Stand	LSKMAGS08
Magna GrIP™ Rack (8 well)	20-400
Magna GrIP™ HT96 Rack (for 96 well plates)	17-10071
Magna ChIP™ Protein A+G Magnetic Beads	16-663

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