A comparative test of magnetic LOABeads™ Protein A and HiTrap® Protein A column chromatography

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Introduction

Magnetic beads coupled with protein A are a popular research tool, as they provide a rapid and convenient method to conduct antibody-based experiments in small scale using simple magnetic separation, instead of more advanced and time-consuming techniques such as chromatography or centrifugation. In contrast to chromatography where porous beads are used, the traditional magnetic beads, such as Dynabeads®, are solid with a limited binding surface area. Recently, porous magnetic beads have been commercialized, which combines the ease of use from a magnetic particle with the high binding capacity of a porous bead. This makes porous magnetic beads an attractive alternative to complex techniques like column chromatography, as well as other adsorption applications where a high capacity is essential.

In this study, we compare the performance of purifying mono- and polyclonal antibodies from ascites fluid or serum, using LOABeads Protein A, a porous magnetic agarose particle, and a handheld cube magnet, versus a HiTrap Protein A column coupled to a chromatography instrument.

In conclusion, LOABeads Protein A has significant advantages such as scalability, flexibility, ease of use, and the absence of complex and expensive instrumentation compared to HiTrap column chromatography. Concerning yield, purity, and functionality of the purified antibodies, the two purification systems perform equally well.

Purification of mono- and polyclonal anti-*Listeria* antibodies

In a first test, monoclonal- (mAb) and polyclonal (pAb) anti-*Listeria* antibodies produced as mouse ascites were purified using LOABeads Protein A combined with a handheld neodymium cube magnet (12 mm) or a HiTrap Protein A column coupled to a chromatography system (see Table 1).

For the magnetic bead based purification, the two antibody samples were purified in parallel. Beads from 500 µl 10% LOABeads Protein A suspension (50 µl beads) were mixed with 1 ml of sample (500 µl of either ascites fluid + 500 µl PBS) for 1 hour. After washing the beads, adsorbed antibodies were eluted from the LOABeads Protein A using 10 bead volumes, 500 µl, of elution buffer.

For the column chromatography technique, the two samples were purified in serial. 500 µl of each mouse ascites sample, diluted with 500 µl of PBS, were purified with the 1 ml HiTrap Protein A column. Elution was performed with 2 column volumes, 2 ml, of elution buffer.

Table 1. Experimental conditions for purification of anti-*Listeria* antibodies.

Supplier	Lab on a Bead	GE Healthcare
Separation medium	LOABeads Protein A	HiTrap Protein A
Binding capacity	40 mg/ml settled beads	30 mg/ml
Separator	Cube magnet	Chromatography system
Sample 1	mAb anti-*Listeria* in 500 µl mouse ascites fluid	
Sample 2	pAb anti-*Listeria* in 500 µl mouse ascites fluid	
Bead volume	50 µl	1 ml
Load	50% of binding capacity	4% of binding capacity
Elution volume	500 µl	2 ml

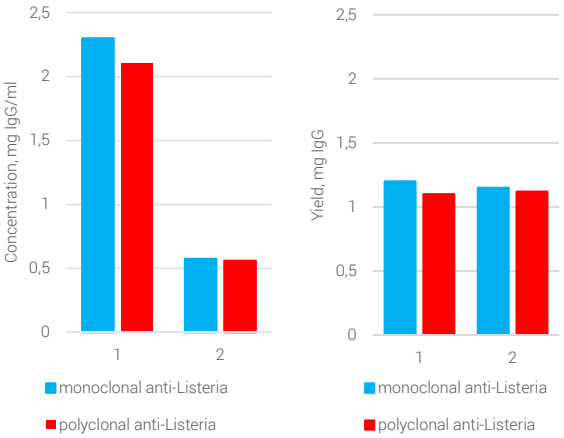

Fig 1. Concentration and yield of purified antibodies from ascites fluid using (1) LOABeads Protein A or (2) HiTrap Protein A.

Concentration and yield of the purified antibodies were determined spectrophotometrically (Fig 1). The antibody concentrations were more than 4 times higher using LOABeads Protein A compared to the HiTrap Protein A column, whereas the yields were approximately the same.

The eluted antibodies were further analyzed by SDS-PAGE under reducing conditions and stained with Coomassie blue in order to evaluate the purity (Fig 2). The purity was consistently high and similar for the two antibodies purified with either magnetic beads or column chromatography.

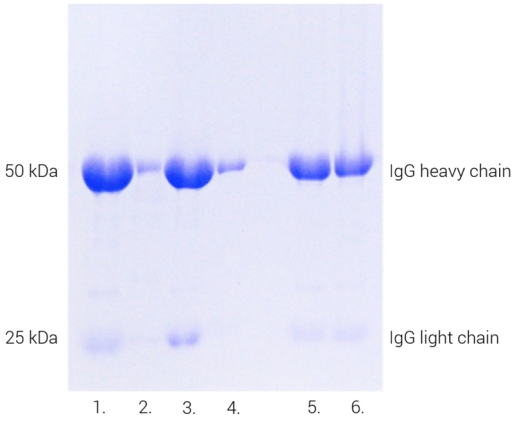

Fig 2. Purity assessment of monoclonal- and polyclonal anti-*Listeria* antibodies purified from mouse ascites fluid using LOABeads Protein A or HiTrap Protein A. SDS-PAGE under reducing conditions and stained with Coomassie blue. Lane 1-2, mAb using LOABeads Protein A first resp. second elution, respectively. Lane 3-4, pAb using LOABeads Protein A first resp. second elution, respectively. Lane 5, mAb using HiTrap Protein A and lane 6, pAb using HiTrap Protein A.

Purification of serum IgG from P166-immunized rabbit

In a second test, LOABeads Protein A and a HiTrap Protein A column were used in the purification of total IgG from serum of a P166-immunized rabbit. 0.5 ml of serum was diluted with 0.5 ml of PBS and applied either to 100 µl settled pre-washed LOABeads or the 1 ml HiTrap column. After elution, the beads were regenerated and reused another 2 times. The amount of purified anti-P166 total IgG antibodies were on average 3.3 mg using LOABeads Protein A and 3.2 mg using HiTrap Protein A (Fig 3). Both methods showed consistent reusability when using the beads 3 times in total.

In order to evaluate the purity, the purified antibodies were analyzed by SDS-PAGE under reducing and non-reducing conditions, using silver and Coomassie blue staining (Fig 4). A high purity was indicated in the eluted fractions, as only bands originating from full length IgG, non-reducing, and the heavy and light chains of IgG, reducing, were visible.

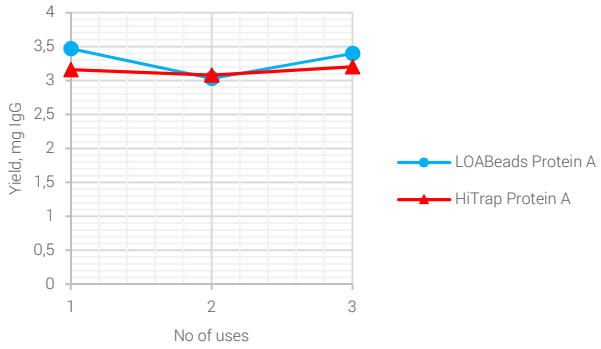

Fig 3. Amount of purified anti-P166 total IgG antibodies from 0.5 ml rabbit serum, using LOABeads Protein A or HiTrap Protein A, in three consecutive purification cycles.

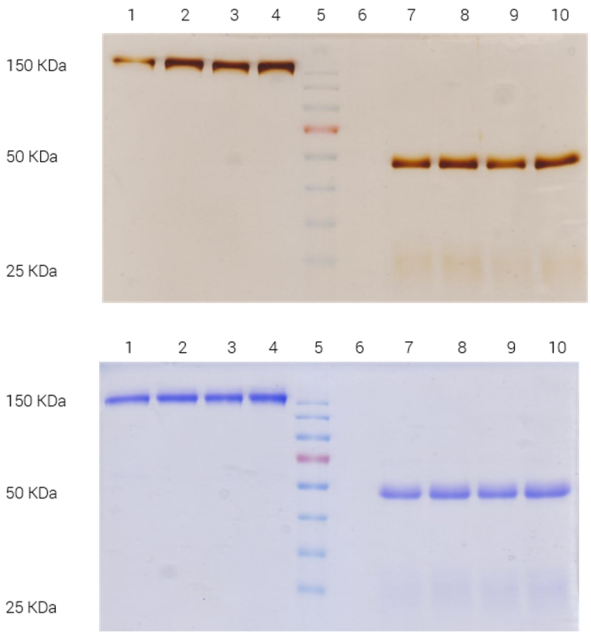

Fig 4. Purity assessment of purified P166 peptide antibodies using SDS-PAGE. Lane 1-2, LOABeads Protein A use no. 1 resp. 2 non-reducing conditions. Lane 3-4, HiTrap Protein A use no. 1 resp. 2 non-reducing conditions. Lane 5, marker. Lane 7-8, LOABeads Protein A use no. 1 resp. 2 reducing conditions. Lane 9-10, HiTrap Protein A use no. 1 resp. 2 reducing conditions. Upper gel, 1 µg/well and silver staining, and lower gel, 5 µg/well and Coomassie blue staining.

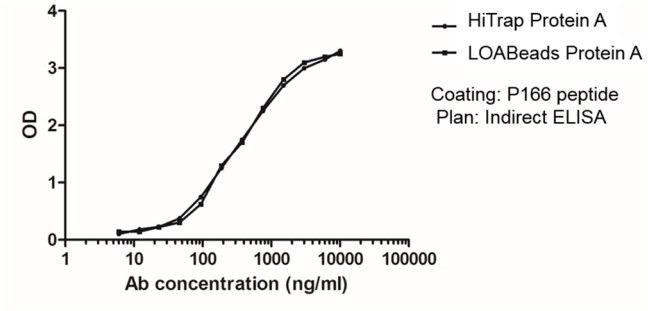

Fig 5. Functional assessment of purified P166 peptide antibodies from rabbit serum using ELISA.

The functionality of the purified anti-P166 total IgG antibodies was further analyzed with ELISA, where no difference in performance could be observed between antibodies purified in either way (Fig 5).

Conclusions

These comparative tests, show that LOABeads Protein A in combination with a neodymium cube magnet has equal performance compared to a HiTrap Protein A column coupled to a chromatography system regarding yield, purity, and functionality of the purified antibodies. Reusability of the beads was demonstrated over 3 purification cycles, with no differences in binding capacity or selectivity observed for the two techniques. Major advantages of working with magnetic beads, compare to column chromatography, are the possibility to work in parallel with several samples (flexibility), the scalability of the amount of beads to be used for purification, ease of use, and absence of complex and expensive instrumentation, which makes magnetic high capacity beads a robust and reliable purification technique. Scalability offers the possibility to match the amount of beads used in a purification setup with the antibody concentration and sample volume in the starting material, in order to maximize the load of binding capacity. The higher concentration of purified antibodies in the magnetic bead eluates, can also be of benefit in various downstream applications.

LOABeads Protein A is a magnetic agarose particle which combines the simplicity of working with magnetic separation techniques, with the high binding capacity properties of porous beads. The performance of the magnetic bead technology is equal or better compared to column chromatography.

Ordering information

Product	Product number
LOABeads Protein A, 2 x 1ml	1001-2ml
LOABeads Protein A, 5 x 1ml	1001-5ml

For more information, visit www.labonabead.se