Affinity purification of disease biomarkers from human serum using LOABeads™ AffiActive10

G. Hjälm[1], K. Lundberg[2], F. Real Fernández[3]*
[1]Lab on a Bead AB, Uppsala, Sweden
[2]Lab on a Bead AB, Gothenburg, Sweden
[3]PeptLab, Dipartimento NeuroFarBa, Sezione di Farmaceutica e Nutraceutica, Universita' di Firenze, Italy

Introduction

The capture of specific biomarkers through protein-protein interactions is one application for affinity purification. Detection of biomarkers can prove very beneficial as a tool for initial diagnosis of a disease, but also to monitor the progress of a disease and, therefore, aid in decision making during therapeutic treatment. The synthetic glycopeptide CSF114(Glc) has been shown to bind autoantibodies as biomarkers involved in the progression of multiple sclerosis (MS) [1]. In this study, CSF114(Glc) was immobilized to LOABeads AffiActive10 and the magnetic beads were tested for their ability to purify autoantibodies present in serum from a patient diagnosed with MS.

Coupling of peptide to magnetic beads

Glycopeptide CSF114(Glc) was dissolved in D-PBS and incubated with LOABeads AffiActive10 beads for three hours (Table 1). The beads were washed twice using binding buffer, before remaining reactive structures were blocked for 45 minutes using ethanolamine (50 volume percent in D-PBS). Final handling of the beads was according to the manufacturer's instructions in the Product Manual. Measuring absorbance at 280 nm on unbound material, showed that approximately 0.15 mg glycopeptide was coupled to 0.1 ml beads, giving a calculated coupling of 1.5 mg per ml bead.

Table 1. Experimental conditions for coupling

Magnetic bead	LOABeads™ AffiActive10
Ligand	Glycopeptide CSF114(Glc)
Bead volume	0.1 ml (1 ml 10% bead suspension)
Sample volume	0.3 mg in 1 ml D-PBS (pH 7.2)
Magnetic separator	LOABeads™ MagSep5
Coupling conditions	D-PBS (pH 7.2), 3 h, RT, shaking

Capture of autoantibodies from MS serum

Human serum was diluted 1:1 with binding buffer and filtered through a 0.22 nm nitrocellulose membrane (Table 2). 2 ml diluted serum was then added to 0.1 ml of settled beads, coupled with peptide as described in the previous section. Sample and beads were mixed for 2 hours at room temperature, after which the supernatant was removed and the beads washed with D-PBS. Elution of antibodies was carried out in two rounds using glycine-HCl (pH 2.5) and a contact time of 15 minutes each. Eluted material was neutralized using 1 M Tris-HCl pH 10 and then concentrated (using AMICON CENTRIPREP 50K MWCO tubes) and recovered in D-PBS.

An ELISA was performed to confirm the presence of glycopeptide CSF114(Glc) reactive IgG and IgM in the eluate (Fig 1). Shortly, an ELISA plate was coated with CSF114(Glc), incubated with eluate, probed with either secondary anti-human IgG-AP or anti-human IgM-AP, after which pNPP was used as substrate [2]. Absorbance at 280 nm reveled a total yield of 0.77 mg CSF114(Glc)-positive antibodies.

Table 2. Experimental conditions for capture

Magnetic beads	LOABeads™ AffiActive10 coupled with CSF114(Glc)
Sample	Patient serum
Bead volume	0.1 ml (1 ml 10% bead suspension)
Sample volume	2 ml (serum diluted 1:1 in D-PBS (pH 7.2) and filtered at 0.22 nm)
Magnetic separator	LOABeads™ MagSep5
Binding conditions	D-PBS (pH 7.2), 2 h, RT, shaking
Elution conditions	2x1 ml 100 mM Gly-HCl* (pH 2.5) 15 min

* 60 mM citrate (pH 3.0) is recommended as a low pH buffer to be used with LOABeads.AffiActive10

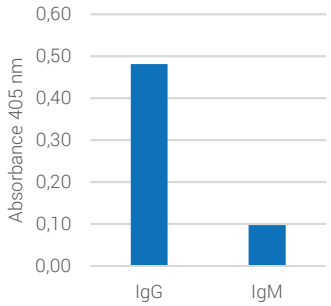

Fig 1. Non-quantitative ELISA of glycopeptide CSF114(Glc) reactive material. Levels are expressed as arbitrary units at absorbance 405 nm

Conclusions

Coupling of the synthetic glycopeptide CSF114(Glc), under physiological and mild conditions, to magnetic LOABeads AffiActive10, provides an efficient strategy to purify autoantibodies from patients with multiple sclerosis. The simple technique of using magnetic separation, as described herein, shows a low cost method, with equal yields as using a chromatography instrument (data not shown), for enrichment of autoantibodies involved in a disease.

References

1 Lolli, F., et al (2005) An N-glucosylated peptide detecting disease-specific autoantibodies, biomarkers of multiple sclerosis. *PNAS* 102, 10273-10278.

2 Lolli, F., et al (2005) The glycopeptide CSF114(Glc) detects serum antibodies in multiple sclerosis. *J Neuroimmunol* 167, 131-137.

*This Application Note has been compiled by employees of Lab on a Bead AB, using original data kindly provided by Dr. Real Fernández. The data has been obtained using a free sample and evaluation of LOABeads™ AffiActive10. No payment for service or consultation have occurred. The final text has been approved by Dr. Real Fernández.