

Protein isolation

Isolation of target protein from mouse brain lysate

Introduction and background

Isolating a specific protein from a complex matrix is one of the applications in which our MagSi-protein A or MagSi-protein G beads can be used. The high paramagnetic strength facilitates a quick and easy separation by using a magnet, and the coupled protein A or protein G on the surface makes it possible to bind your own antibody.

Experiment

Mouse brain lysate was produced by homogenization using a glass teflon homogenizer (in 1xPBS plus protease inhibitors (PI)) and cell debris were removed gently by centrifugation. 5 mg mouse brain lysate was incubated with 25 μ l (10 mg/ml) MagSi protein A or -protein G beads coupled with antibody. As reference, 50 μ l (10 mg/ml) of competitor protein A or protein G beads were used. This was mixed by gentle rotating at room temperature for 4 hours. The supernatant was discarded by a magnetic device and the beads with the captured proteins/organelles were washed 3x with 1x PBS plus PI, the supernatant was always discarded by a magnetic device. Finally, the probe was prepared for SDS-PAGE by denaturation.

Two antibodies were coupled to the protein A or protein G beads to capture 2 different antigens. Antigen 1 was APLP1 (polyclonal ab), Antigen 2 was a kinesin subunit (monoclonal ab).

Results

The SDS PAGE results in figure 1 show the isolated protein. High amount of protein and only very little other bands (background absorption) are visible. Lower amounts of MagSi beads were used because of the higher binding capacity as compared to the competitor beads.

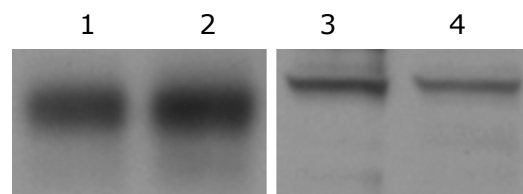


Figure 1: SDS PAGE gel.

1: 25 μ l MagSi-Protein A, 2: 50 μ l competitor protein A,
3: 25 μ l MagSi-Protein G, 4: 50 μ l competitor protein G

Conclusion

The MagSi-Beads can isolate a protein from a difficult matrix like a brain lysate with high recovery and relatively low background absorption.

Acknowledgements

Dr. Szodorai of the University of Heidelberg for the experiments.