

# Immunoprecipitation of human B cell surface antigens using Protein A MicroBeads

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## Introduction

The detection of cell surface antigens by immunoprecipitation and subsequent Western blot analysis is often hampered by technical difficulties, such as insufficient lysis of the proteins from the cell membrane fractions or a high background of unspecifically precipitated proteins. Here, we describe the immunoprecipitation of the MHC class I antigen and the human B cell surface antigen, CD22, using Protein A MicroBeads. The immunoprecipitated proteins were clearly detectable by Western blotting without background staining.

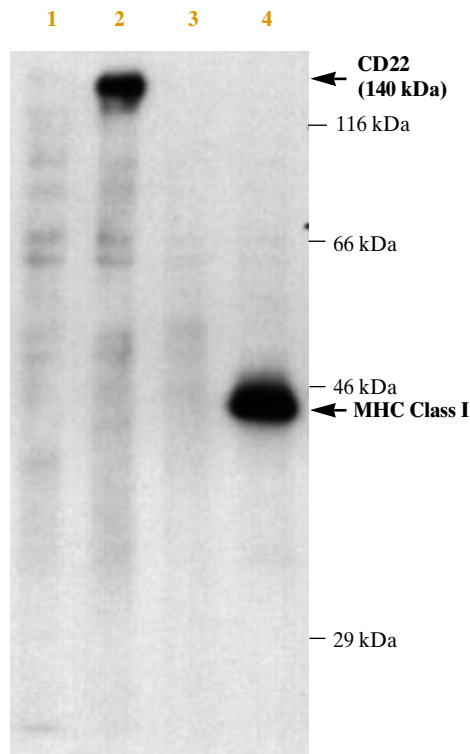
## Materials and methods

### Biotinylation of surface antigens of JOK-1 cells

The surface proteins of a lymphoblastoid B cell line, JOK-1, were biotinylated using EZ-link-sulfo-NHS biotin according to the manufacturer's instructions (Pierce, IL, USA).

### Cell lysis and immunoprecipitation

1x10<sup>7</sup> biotinylated JOK-1 cells were lysed in 150 mM NaCl, 50 mM Tris (pH 8), 1% Triton X-100 supplemented with proteinase inhibitors (protease inhibitor cocktail, Roche, Mannheim, Germany) for 20 minutes on ice, occasionally mixing. For immunoprecipitation with MACS, 400 µl cell lysate were incubated with 2 µg specific antibody (MHC class I: clone W6/32 [2], own production; CD22: clone HD239 [3], own production) and 50 µl Protein A MicroBeads. After mixing, the lysate was incubated for 30 minutes on ice.

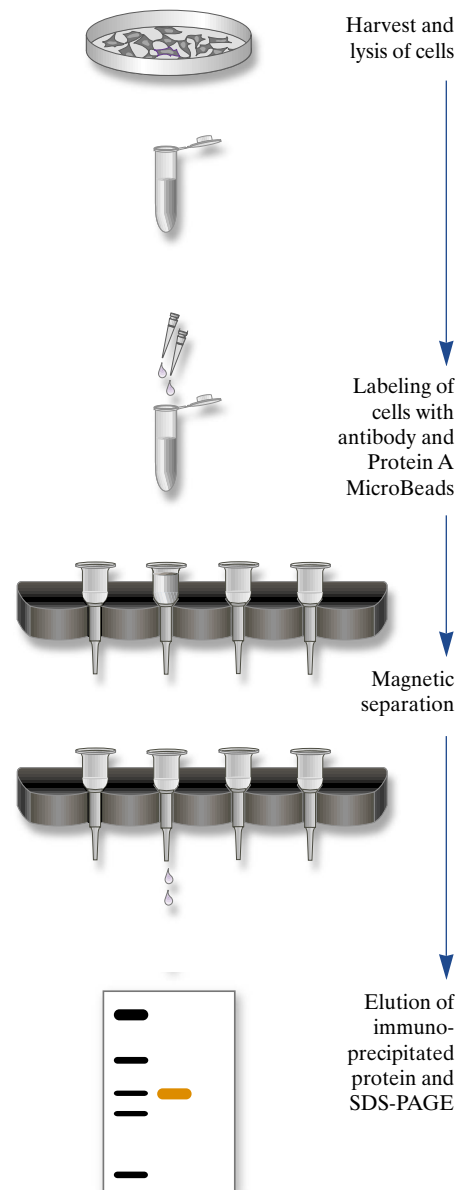


### Western blot of immunoprecipitated surface antigens of the B cell line JOK-1.

For immunoprecipitation either a control antibody as an isotype control (lane 1 and 3) was used or the specific antibodies HD239 (lane 2) or W6/32 (lane 4) were used.

For magnetic immunoprecipitation, a µ Column was placed in the magnetic field of a µMACS separator. The column was prepared by rinsing it with 200 µl lysis buffer. Then the lysate was applied onto the column. When the lysate had run through completely, the µ Column was washed with

## Immunoprecipitation using Protein A MicroBeads



4x200 µl wash buffer (150 mM NaCl, 50 mM Tris-HCl (pH 8), 1% NP-40, 0.1% DOC, 0.5% SDS). Subsequently, the column was rinsed with low salt wash buffer (20 mM Tris/HCl pH 7.5) to remove residual salt which may impair SDS-PAGE (SDS polyacrylamide gel electrophoresis).

For elution, 20 µl of 1x SDS-PAGE sample buffer (Roti®-Load 1, Roth, Karlsruhe, Germany) were added, incubated for 5 minutes and the immunoprecipitated proteins were eluted by adding a further 50 µl of elution buffer.

#### SDS-PAGE and Western blotting

For SDS-PAGE, the eluted proteins were incubated at 95°C for 5 minutes. The proteins were analyzed on a continuous 10% SDS-PAGE. The electrophoresed proteins were blotted on a FluoroTrans 0.2 µm PVDF membrane (Pall Gelman Sciences, Ann Arbor, MI, USA) which was incubated for 30 minutes with Streptavidin-peroxidase at a ratio of 1:100,000. The membrane was washed 3x with PBS. The immunoprecipitated proteins were visualized chemiluminescently by incubating the membrane with super signal west dura extended duration substrate (Pierce, Rockford, IL, USA) for 5 minutes.

The ECL signal was detected on an X-ray film (BIOMAX-MR, Kodak, Rochester, NY, USA).

#### Results and discussion

The two surface proteins, MHC class I and CD22, which are expressed on the human B cell line JOK-1, could be magnetically immunoprecipitated as visualized by Western blotting. The sensitivity achieved was comparable to that which is usually achieved with an immuno-precipitation of <sup>125</sup>I radioactively labeled surface antigens.

The use of Protein A MicroBeads offered a much quicker and simpler procedure than conventional immunoprecipitations with Protein A sepharose. While a conventional immunoprecipitation usually lasts more than one day, immunoprecipitation with Protein A MicroBeads was completed in 1-2 hours. Magnetic immunoprecipitation had the advantage that pre-absorption steps (in total two) and the overnight immunoprecipitation could be omitted. Non-specifically bound material was effectively removed during the wash steps with the µ Column. A 30 minute incubation

with antibody and beads was sufficient for efficient magnetic labeling the surface antigens.

In our opinion, the time-saving aspect of using Protein A MicroBeads may well justify slightly higher costs as manpower is a cost factor which should not be neglected. Taken together, Protein A MicroBeads offered a very quick and efficient alternative to conventional Protein A sepharose. The column technology allowed very convenient and rapid wash steps which may be of special importance when performing highly specific immunoprecipitations such as immunoprecipitations of surface antigens. Further applications for Protein A MicroBeads could be, co-immunoprecipitations and isolation of signal transduction complexes with subsequent signal transduction studies.

#### References

1. Ausubel *et al.* (1987) *Curr. Protocols in Mol. Biol.*, John Wiley & Sons, New York.
2. Barnstable *et al.* (1978) *Cell* 14: 9-20.
3. Schwartz-Albiez *et al.* (1991) *Int. Immunol.* 3: 623-633.

#### Product Information

MAGmol Protein A MicroBeads, 2 ml, for 20-40 immunoprecipitations	Order No. 710-01
MAGmol Protein G MicroBeads, 2 ml, for 20-40 immunoprecipitations	Order No. 711-01
µMACS Starting Kit, includes 2 ml MAGmol MicroBeads, 20 µ Columns, 1 µMACS Separator, 1 MultiStand	Order No. 426-01
µ Columns, 20 pcs.	Order No. 427-01

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