β1 Integrin Cell-surface Immunoprecipitation (Selective Immunoprecipitation)

Ralph T. Böttcher*

Molecular Medicine Division, Max Planck Institute for Biochemistry, Martinsried, Germany
*For correspondence: rboettch@biochem.mpg.de

[Abstract] Immunoprecipitation (IP) is a widely used method to isolate a specific protein from a mixed protein sample using an antibody that exclusively binds to that particular protein. This technique allows studying protein-protein and protein-nucleic acid interactions or to identify post-translational protein modifications. Many proteins, in particular cell surface receptors, localize to different compartments within cells where they elicit distinct functions by interacting with specific proteins. Integrins represent a major family of cell surface receptors consisting of non-covalently associated α and β subunits that mediate the interaction of cells with their environment. However, integrins do not only localize to the cell surface but are also present in other compartments including the endoplasmic reticulum and endosomes where they engage with a distinct set of interacting partners or show distinct post-translational modifications. Standard immunoprecipitation of β1 integrins from a cell lysate without prior fractionation isolates β1 integrins from all compartments. In contrast, selective immunoprecipitation of cell surface β1 integrin allows enriching for the pool of β1 integrin on the cell surface thereby minimizing contaminations with β1 integrins from other subcellular compartments. To achieve this, living cells are incubated with a β1 integrin-specific antibody on ice to label cell surface β1 integrins prior to cell lysis and precipitation.

Materials and Reagents

1. Mouse fibroblasts lacking β1 integrin (β1 -/-) or re-expressing wild-type β1 integrin (β1 wt)
   Note: These cells are home-made immortalized mouse fibroblasts derived from floxed β1 parental cells. β1 -/- cells are used as negative control. However, the protocol can also be transferred to other mouse cell lines. When cells lacking β1 integrin are not available as negative control one has to include an unrelated antibody (see steps 2 and 4) to monitor for unspecific binding.
2. Dulbecco's Modified Eagle's Medium (DMEM) with GlutaMAX-I (Life Technologies, Gibco, catalog number: 31966-021)
3. Fetal Bovine Serum (FBS) (PAA, catalog number: A15-101)
4. PBS (Sigma-Aldrich, catalog number: P4417)
5. 0.5% Trypsin/EDTA (Life Technologies, Gibco®, catalog number: 15400-054)

6. Primaquine bisphosphate (Sigma-Aldrich, catalog number: 160393)
7. Protein G sepharose (Protein G sepharose Fast Flow) (Sigma-Aldrich, catalog number: P3296)
8. BCA Protein Assay (Thermo Fisher Scientific, catalog number: 23227)
9. Triton X100
10. Tris-HCl
11. Na-deoxycholate
12. SDS
13. Glycerol
14. Bromphenol blue
15. Mercapthoethanol
16. Protease inhibitors (Complete Mini EDTA-free) (Roche, catalog number: 04 693 159 001)
17. Phosphatase inhibitors (Phosphatase Inhibitor Cocktail 2 and 3) (Sigma-Aldrich, catalog numbers: P5726 and P0044)
18. Talin-1 antibody (1:1,000 for western blotting) (Sigma-Aldrich, catalog number: T3287)
19. SNX17 antibody (1:1,000 for western blotting) (Proteintech, catalog number: 10275-1-AP)
20. β1 integrin IP buffer (see Recipes)
21. 2x Laemmli sample buffer (see Recipes)

Equipment

1. Cell scraper
2. 10 cm cell culture dish
3. Centrifuge
4. 37 °C, 5% CO_2 cell culture incubator
5. 26-G needle attached to 1-ml syringe
6. Heating block (Eppendorf Thermomixer compact or equivalent)

Procedure

1. Wash mouse fibroblasts expressing β1 integrin (β1 wt) and fibroblasts lacking β1 integrin (β1 -/-) with PBS, trypsinize and count cells using Glass slide with grids.
2. For both cell lines plate 2 x 10^6 cells per 10 cm dish and incubate in DMEM/10%FBS in the 5% CO_2 incubator at 37 °C overnight.
   Note: We used the cell line lacking β1 integrin as negative control. Alternatively, one can plate cells expressing β1 integrin for incubation with an unrelated control antibody (step 4).

3. Place dishes on ice and wash twice with ice-cold PBS (4 ml/dish).
4. To label cell surface β1 integrin, incubate the cells in 3 ml ice-cold DMEM/10%FBS containing the anti-β1 integrin antibody or an unrelated antibody as negative control.
   Note: Anti-β1 integrin antibody: The antibody has to be directed against an epitope in the extracellular domain of integrin and has to recognize β1 integrin in its native conformation. We used a home-made antibody against mouse β1 integrin in a concentration of 1:1,500 (Böttcher et al., 2012). For other antibodies the amount has to be determined experimentally.
   Control antibody: Should be derived from the same species as the anti-β1 integrin antibody and should be used in the same concentration.
5. Place dishes on rocker (approximately 7 see-saw movements per minute) at 4 °C and incubate for 60 min.
6. Place dishes on ice and wash twice with ice-cold PBS (4 ml/dish).
   To selectively immunoprecipitate cell surface β1 integrin continue with step 9.
7. To immunoprecipitate β1 integrin from the endosomal compartment, incubate the cells in DMEM/10%FBS containing primaquine for 15 min at 37 °C.
   Note: After antibody binding to cell surface β1 integrins on ice it is possible to induce β1 integrin endocytosis by incubating the cells at 37 °C. This enables the antibody-β1 integrin complexes to reach the endosomal compartment. β1 integrins are rapidly internalized and the addition of primaquine inhibits recycling of β1 integrin from endosomes back to the cell surface thereby enriching the amount of β1 integrin in endosomes. Depending on the cell type, 0.6 μM to 0.6 mM primaquine are used.
8. Place dishes on ice and wash once with ice-cold PBS (4 ml/dish).
9. Lyse cells in 1 ml β1 integrin IP buffer per 10 cm dish for 15 min on ice.
10. Scrape off cells and transfer the cell lysate into pre-cooled 1.5 ml reaction tubes.
11. Sonicate briefly or pass several times through a 26 gauge needle.
12. Spin cell lysate at 17,000 *x g* for 10 min at 4 °C.
13. Transfer the supernatant into fresh pre-cooled 1.5 ml reaction tubes.
14. Take out 60 µl for whole cell lysate sample and 5.0 µl to determine the protein concentration.
15. Take 30.0 µl Protein G sepharose slurry per 1 mg protein, wash three times with β1 integrin IP buffer and add equal amount of the cell lysis supernatant per sample (between 1.0-1.5 mg cell lysate was used per sample).
16. Incubate for 2 h at 4 °C on a rocking platform or a rotator.
17. Spin the Eppendorf tube at 1,500 *x g* for 2 min at 4 °C. Remove the supernatant completely and wash the beads 3-5 times with 500 µl of β1 integrin IP buffer.

18. After the last wash, take off supernatant and elute proteins by heating to 90-100 °C for 7 minutes in 60 µl of 2x Laemmli sample buffer.
19. Spin at 10,000 *x g* for 30 sec, collect supernatant and load onto the gel. Supernatant samples can be collected and kept frozen at this point if the gel is to be run later.

    Note: β1 integrin translation/processing is a tightly controlled step-wise process that starts with the synthesis of a 88 kDa polypeptide that undergoes sequential glycosylation in the ER ('immature' form 105 kDa) and in the Golgi giving rise to incompletely glycosylated β1 integrin subunit and a complete or 'mature' β1 subunit of around 125 kDa.

    A successful immunoprecipitation of the cell-surface β1 integrins can be shown by western blotting with an antibody against β1 integrin (e.g. the antibody used for immunoprecipiation; dilution 1:10,000). The immature 105 kDa β1 integrin should be strongly reduced, ideally not detectable, after the immunoprecipitation. To further characterize the purity of your cell surface β1 integrins, the precipitate can be analyzed by western blotting for co-immunoprecipitated proteins such as talin-1 (interacts with β1 integrin at the plasma membrane; 1:1,000 for western blotting) or SNX17 (interacts with β1 integrin on endosomes; 1:1,000 for western blotting).

Recipes

1. β1 integrin IP buffer
   50 mM Tris-HCl (pH 7.5)
   150 mM NaCl
   1% Triton X100
   0.1% Na-deoxycholate
   1 mM EDTA
   Protease inhibitors (Complete Mini EDTA-free; 1 tablet for 10 ml of buffer)
   Phosphatase inhibitors (Phosphatase Inhibitor Cocktail 2 and 3; 1:100 dilution from stock)
2. 2x Laemmli sample buffer
   120 mM Tris-HCl (pH 6.8)
   4% SDS
   20% glycerol
   4 mM EDTA
   0.001% bromphenol blue
   2% mercapthoethanol

Acknowledgments

This protocol was adapted from a paper by Böttcher *et al.* (2012). We thank R. Fässler for critically reading the manuscript and continuous support. This work was funded by the Deutsche Forschungsgemeinschaft (SFB 914, project A05).

References

1. Böttcher, R. T., Stremmel, C., Meves, A., Meyer, H., Widmaier, M., Tseng, H. Y. and Fässler, R. (2012). Sorting nexin 17 prevents lysosomal degradation of β1 integrins by binding to the β1-integrin tail. *Nat Cell Biol* 14(6): 584-592.

