Protein Production & Assays

For research use only

Protocol

Immunoprecipitation with Selector Resins

1 DESCRIPTION

Selector Resins for easy and efficient immunoprecipitation of fluorescent fusion proteins (e.g GFP, RFP,…) are based on high-affinity single-domain antibody (sdAb) fragments derived from lamas or alpacas. This type of affinity tags (also known as Nanobodies®) provides significant advantages over conventional IgG molecules with respect to affinity and specificity, which leads to a low signal:noise ratio and high protein yields. The Selector Resins consist of high-affinity single-domain antibody (sdAb) fragments that are covalently immobilized on 4% cross-linked agarose beads. The innovative, oriented, and selective attachment via flexible linkers guarantees optimal accessibility of the sdAbs and in addition largely eliminates batch-to-batch variations. Due to the single-chain nature of sdAbs and their stable and covalent attachment, no leakage of light and heavy chains is observed during elution with SDS sample buffer. Selector Resins thus feature high affinity and superior capacity for fusion proteins while showing negligible non-specific background. Selector Resins are compatible with physiological or high stringency buffers (see product-specific compatibility charts) and reducing agents.

2 AVAILABLE SELECTOR RESINS

Selector Resins are originally manufactured by NanoTag Biotechnologies GmbH. They are intended for research applications, not for diagnostic or therapeutic use! Each item contains 2000 µl resin sufficient for approximately 100 reactions. Following Selector Resins are provided:

- MBP Selector (Cat. No. 2-9111-020)
- GST Selector (Cat. No. 2-9121-020)
- GFP Selector (Cat. No. 2-9131-020)
- RFP Selector (Cat. No. 2-9141-020)
- TagFP Selector (Cat. No. 2-9151-020)

The application of Selector Resins in combination with Mini Spin Columns (Cat. No. 2-9102-050) is recommended for most efficient washing and elution. However, batch purification is possible as well (see protocol below).

3 INITIAL PREPARATIONS

Selector Resins are compatible with most common lysis and washing buffers, e.g., RIPA. For custom buffers please refer to the product-specific compatibility chart. For the folllwoing protocol the preparation of Tris-buffered saline (TBS) pH 7.4 and 2x SDS sample buffer is necessary.

4 PROTOCOL

4.1 Immunoprecipitation using Mini Spin Columns

4.1.1 Prepare native cell lysates (0.2 to 1.5 ml volume) according to established protocols. For mammalian cells, we recommend using 1 x 10^6- 1 x 10^8 cells per experiment.

4.1.2 Clarify lysate by centrifugation for 10 min at > 14000 x g and 4 °C. Take sample for further analysis (input fraction).

4.1.3 Equilibration of the Selector Resins:
Resuspend Selector Resin and transfer 20 µl slurry (10 µl packed beads) into a clean 1.5 ml reaction tube. Add 1 ml lysis buffer and centrifuge for 1 min at 1000 x g. Carefully remove supernatant. Repeat this step.

4.1.4 Add clarified lysate to equilibrated Selector Resin.

4.1.5 Incubate 1 h at 4 °C with head-over-tail rotation.

4.1.6 Sediment beads by centrifugation for 1 min at 1000 x g and 4 °C. Take sample from supernatant for further analysis (non-bound fraction).

4.1.7 Wash of the Selector Resin:
Carefully remove the supernatant and resuspend the beads in 1 ml lysis buffer. Centrifuge for 1 min at 1000 x g and remove the supernatant.

4.1.8 Transfer to Mini Spin Column:
Remove bottom plug from Mini Spin Column. Place column in 2 ml reaction tube. Resuspend beads in 200 µl lysis buffer. Transfer suspension to Mini Spin column. Wash out beads sticking to tube with 200 µl lysis buffer and transfer to column. Centrifuge column for 1 min at 1000 x g, discard flow-through.

4.1.9 Wash twice with 400 µl wash buffer, centrifuge for 1 min at 1000 x g.

4.1.10 Wash once with 400 µl TBS, centrifuge for 1 min at 3000 x g.

4.1.11 Attach bottom plug and place Mini Spin Column in a clean 1.5 ml reaction tube.

4.1.12 Resuspend Selector Resin in 50 µl 2x SDS sample buffer.

4.1.13 Heat Mini Spin Column to 95 °C for 2 min.

4.1.14 Remove bottom plug and centrifuge for 1 min at 3000 x g. Boil collected eluate for 5 min at 95 °C and analyze by SDS-PAGE.

4.2 Immunoprecipitation from batch

4.2.1 Prepare native cell lysates (0.2 to 1.5 ml volume) according to established protocols. For mammalian cells, we recommend using 1 x 10^6- 1 x 10^8 cells per experiment.

4.2.2 Clarify lysate by centrifugation for 10 min at > 14000 x g and 4 °C. Take sample for further analysis (input fraction).

4.2.3 Equilibration of the Selector Resins:
Resuspend Selector Resin and transfer 20 µl slurry (10 µl packed beads) into a clean 1.5 ml reaction tube. Add 1 ml lysis buffer and centrifuge for 1 min at 1000 x g. Carefully remove supernatant. Repeat this step.

4.2.4 Add clarified lysate to equilibrated Selector Resin.

4.2.5 Incubate 1 h at 4 °C with head-over-tail rotation.

4.2.6 Sediment beads by centrifugation for 1 min at 1000 x g and 4 °C. Take sample from supernatant for further analysis (non-bound fraction).

4.2.7 Wash of the Selector Resin:
Carefully remove the supernatant and resuspend the beads in 1 ml lysis buffer. Centrifuge for 1 min at 1000 x g and remove the supernatant. Wash beads 2-3 times with wash buffer and then once with TBS

4.2.8 Transfer beads in clean 1.5 ml reaction tube.

4.2.9 Centrifuge for 1 min at 3000 x g.

4.2.10 Carefully and completely remove supernatant.

4.2.11 Resuspend Selector Resin in 50 µl 2x SDS sample buffer.

4.2.12 Heat to 95 °C for 5 min.

4.2.13 Centrifuge for 1 min at 3000 x g. Collect supernatant and analyze by SDS-PAGE.

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If you have any questions, please contact

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We are here to help!