NanoTag Biotechnologies

# Immunoprecipitation Protocol

## Immunoprecipitation of fusion proteins from cell extracts using NanoTag's Selector Resins

NanoTag's Selector Resins are based on high-affinity single-domain antibody (sdAb) fragments 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 not only with physiological buffers but also with high stringency buffers (see product-specific compatibility charts) and reducing agents. Selector Resins thus provide great freedom to adjust the binding and washing conditions to the experimental needs.

**Only for research applications, not for diagnostic or therapeutic use!**

### Products

| | | |
|---|---|---|
| **MBP Selector** | N0110 | 2000 µL slurry (1000 µL resin) |
| **GST Selector** | N0210 | 2000 µL slurry (1000 µL resin) |
| **GFP Selector** | N0310 | 2000 µL slurry (1000 µL resin) |
| **RFP Selector** | N0410 | 2000 µL slurry (1000 µL resin) |
| **TagFP Selector** | N0510 | 2000 µL slurry (1000 µL resin) |

### Materials (not included):

- Lysis buffer[1]
- Washing buffer[1]
- Tris-buffered saline (TBS) pH 7.4
- 2x SDS sample buffer

### Remarks

[1] Selector resins are compatible with most common Lysis/Washing buffers (e.g. RIPA). For custom buffers please refer to the product-specific compatibility chart.

**For further information concerning this protocol please contact us at info@nano-tag.com**

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# Immunoprecipitation Protocol

## Protocol using Mini Spin Columns

**We recommend using Mini Spin Columns (Cat. No. A1001) for immunoprecipitation experiments. An alternative batch protocol is found on the following page.**

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

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

3. Equilibrate GFP Selector Resin
   a. Resuspend GFP Selector Resin.
   b. Transfer 20 µl slurry (10 µl packed beads) into a clean 1.5 ml reaction tube.
   c. Add 1 ml lysis buffer.
   d. Centrifuge for 1 min at 1000 x g and carefully remove supernatant.
   e. Repeat steps c-d once.

4. Add clarified lysate from step 2 to equilibrated GFP Selector Resin obtained in step 3.

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

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)

7. Washing
   a. Carefully remove supernatant.
   b. Resuspend beads in 1 ml Lysis buffer
   c. Centrifuge for 1 min at 1000 x g.
   d. Remove Supernatant

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

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

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

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

12. Resuspend GFP Selector resin in 50 µl 2x SDS sample buffer

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

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.

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# Immunoprecipitation Protocol

## Batch protocol

**Due to the more effective washing steps, we recommend using Mini Spin Columns (Cat. No. A1001) for immunoprecipitation experiments (see protocol on page 2).**

1. Prepare native cell lysates (0.2 to 1.5 ml volume) according to established protocols. For mammalian cells, we recommend using $10^6$-$10^8$ cells per experiment.
2. Clarify lysate by centrifugation for 10 min at > 14000 x g and 4 °C.
   Take sample for further analysis (Input fraction)
3. Equilibrate GFP Selector Resin
   a. Resuspend GFP Selector Resin.
   b. Transfer 20 µl slurry (10 µl packed beads) into a clean 1.5 ml reaction tube.
   c. Add 1 ml lysis buffer.
   d. Centrifuge for 1 min at 1000 x g and carefully remove supernatant.
   e. Repeat steps c-d once.
4. Add clarified lysate from step 2 to equilibrated GFP Selector Resin obtained in step 3.
5. Incubate 1 h at 4 °C with head-over-tail rotation.
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)
7. Washing
   a. Carefully remove supernatant.
   b. Resuspend beads in 1 ml Lysis buffer
   c. Centrifuge for 1 min at 1000 x g.
   d. Wash beads 2-3 times with Washing buffer.
   e. Wash beads once with TBS
8. Transfer beads in clean 1.5 ml reaction tube.
9. Centrifuge for 1 min at 3000 x g.
10. Carefully and completely remove supernatant
11. Resuspend GFP Selector resin in 50 µl 2x SDS sample buffer
12. Heat for 5 min to 95 °C.
13. Centrifuge for 1 min at 3000 x g.
    Collect supernatant and analyze by SDS-PAGE.