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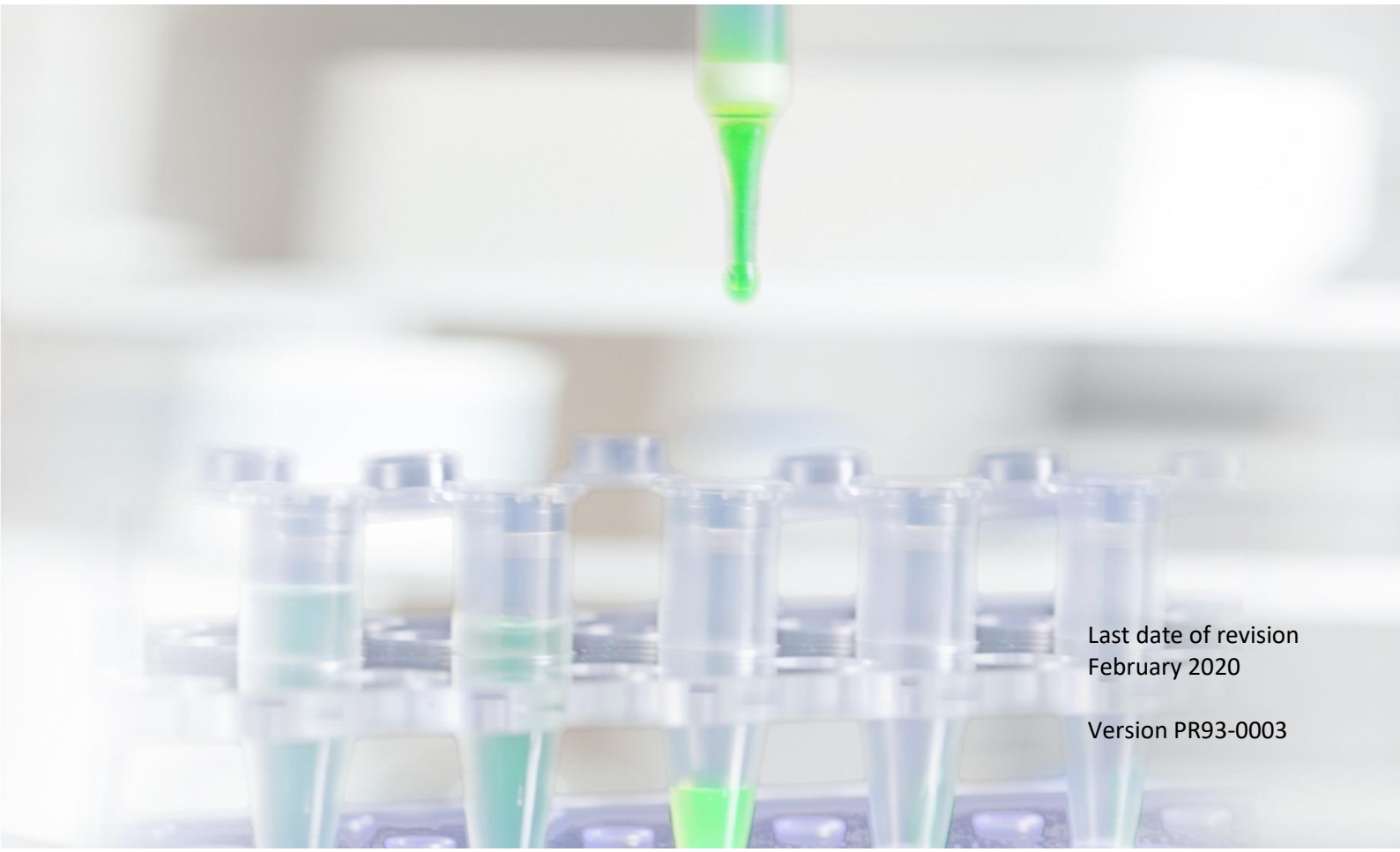
Solutions
For Life Sciences

Protein Production &
Assays

Immunoprecipitation of fusion proteins from cell extracts using Selector Resins

Protocol



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Content

Manuals are available for download under
www.iba-lifesciences.com/download-area.html.

1 Introduction

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 not only with physiological buffers but also with high stringency buffers (see product-specific compatibility charts) and reducing agents. Selector Resins therefore provide great freedom to adjust the binding and washing conditions to experimental needs.

2 Available Selector Resins (Only for research applications, not for diagnostic or therapeutic use!)

Product	Cat. No.
	2000µl (100 reactions)
MBP Selector	2-9111-020
GST Selector	2-9121-020
GFP Selector	2-9131-020
RFP Selector	2-9141-020
TagFP Selector	2-9151-020

Selector Resins are originally manufactured by NanoTag Biotechnologies GmbH.

3 Immunoprecipitation (IP) Protocol

Recommended Buffers/Solutions
Lysis buffer*
Wash buffer*
Tris-buffered saline (TBS) pH 7.4
2x SDS sample buffer

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

Important notes	The use of Mini Spin Columns (Cat. No. 2-9102-050) is recommended for most efficient washing and elution. See 3.2 for an alternative batch protocol.

3.1 IP using Mini Spin Columns

Protocol

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 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 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).

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7. Wash
    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 Wash 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 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.

3.2 IP from batch

Protocol

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 Selector Resin
    a. Resuspend 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 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. Wash

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 Wash 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 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.

For further information concerning this protocol please contact us at selector-support@iba-lifesciences.com

Please download always an up-to-date version of this protocol from:
www.iba-lifesciences.com/download-area.html.

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