

ABIN7529450

RFP-Catcher (agarose magnetic) High-affinity anti-RFP Single-Domain Antibody (sdAb) Protocol

For research use only
Not for use in clinical diagnostic procedures
Version Mar 2024

Catcher Product Line
GFP-Catcher - ABIN5311508
GFP-Catcher - ABIN7272855 Magnetic Beads
RFP-Catcher - ABIN5311510
RFP-Catcher - ABIN7529450 Magnetic Beads
BFP-Catcher - ABIN5311512
GST-Catcher - ABIN5311506
MBP-Catcher - ABIN7272855
mNeonGreen-Catcher - ABIN7529451

Step-by-step Protocol

I. Cell Collection & Lysis

1. For mammalian cells, harvest 10^6 - 10^8 cells per sample.
2. Lyse cells according to established protocols in 0.2 to 1.5 mL volume. Buffer recommendations:

2% Triton X-100, 1% Tween-20, 1% NP-40, 1% CHAPS, 1% Deoxycholate, 0.1% SDS

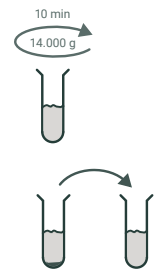
4 M NaCl, 2 M KCl, 1 M MgCl₂, 100 mM EDTA

4 M urea

10 mM DTT, 10 mM 2-Mercaptoethanol

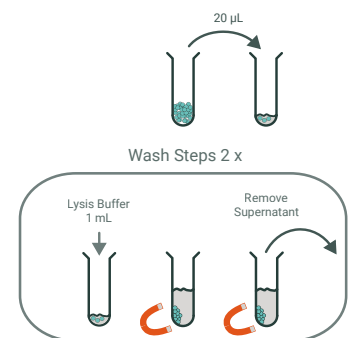
RNAse A, DNase I, Benzonase, protease inhibitors

3. Centrifuge cell lysates in microcentrifuge tubes for 10 min at 14.000 x g at 4°C. Keep a small samples as “input” fraction.
4. Transfer the supernatant to a fresh microcentrifuge tube for each sample and keep at 4 °C.



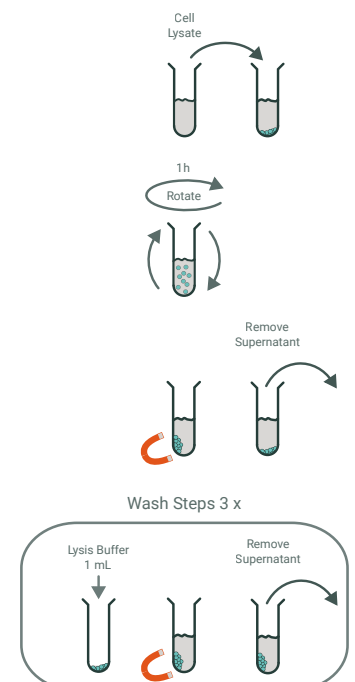
II. Bead Preparation for RFP Capture

5. Homogenize the RFP-Catcher (agarose magnetic beads) slurry gently by shaking.
6. Transfer 20 µL bead slurry to a 1.5 mL microcentrifuge tube for each sample.
7. Add 1 mL Lysis Buffer to equilibrate RFP-Catcher (agarose magnetic beads).
8. Place the tubes on a magnet stand until the fluid is clear. Remove the supernatant carefully.
9. Repeat wash steps once for a total of two washes.



III. Bead Incubation with Supernatant

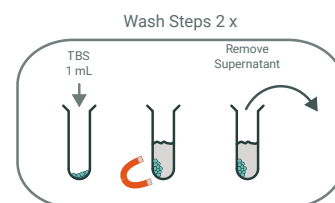
10. Resuspend equilibrated RFP-Catcher (agarose magnetic beads) gently with the cell lysate supernatant.
11. Rotate the microcentrifuge tubes for 1 h at 4 °C.
12. Place the tubes on a magnet stand until the fluid is clear. Keep a small aliquot of the supernatant as “unbound” fraction. Remove the supernatant carefully.
13. Resuspend RFP-Catcher (agarose magnetic beads) in 1 mL Lysis Buffer.
14. Place the tubes on a magnet stand until the fluid is clear. and and carefully remove the supernatant.
15. Repeat wash steps twice for a total of three washes.



Step-by-step Protocol

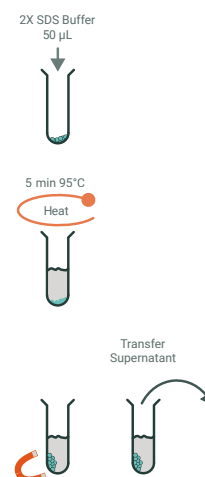
IV. Bead Washing and Solution Changes

16. Resuspend RFP-Catcher (agarose magnetic beads) gently in 1 mL TBS.
17. Place the tubes on a magnet stand until the fluid is clear and carefully remove the supernatant.
18. Repeat wash steps once for a total of two washes.



V. Elution Preparation

19. Resuspend RFP-Catcher (agarose magnetic beads) resin in 50 μ L 2X SDS sample buffer.
20. Heat sample (agarose magnetic beads) resin for 5 min to 95 $^{\circ}$ C.
21. Place the tubes on a magnet stand until the fluid is clear and transfer the supernatant to fresh microcentrifuge tubes. Keep the pellet (agarose magnetic beads) as backup.



For more information, please contact:

antibodies-online Inc.

PO Box 5201
Limerick, PA 19468
USA

Website: www.antibodies-online.com
Email: info@antibodies-online.com
Phone: +1 877 302 8632
Fax: +1 888 205 9894

antibodies-online GmbH

Schloss-Rahe-Straße 15
52072 Aachen
Deutschland

Website: www.antikoerper-online.de
Email: info@antikoerper-online.de
Phone: +49 (0)241 95 163 153
Fax: +49 (0)241 95 163 155