antibodies-online™

ABIN5311508

GFP-Catcher High-affinity anti-GFP 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

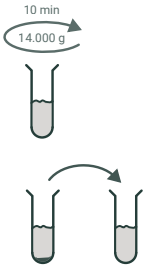
4 M NaCl, 2 M KCl, 1 M MgCl2, 100 mM EDTA

4 M urea

10 mM DTT, 10 mM 2-Mercaptoethanol

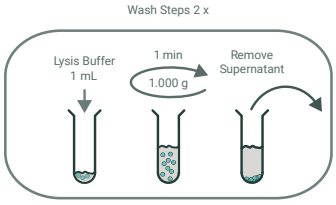
RNAse A, DNAse I, Benzonase, protease inhibitors

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.



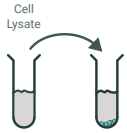
II. Bead Preparation for GFP Capture

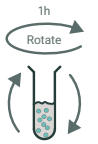
5. Homogenize the GFP-Catcher (agarose beads) slurry gently by shaking.
6. Transfer 20 µL bead slurry to a 1.5 mL microcentrifuge tube for each sample.
7. Add 1 mL Lysis Buffer to equilibrate GFP-Catcher (agarose beads).
8. Centrifuge GFP-Catcher (agarose beads) for 1 min at 1000 x g and carefully remove the supernatant.
9. Repeat wash steps once for a total of two washes.

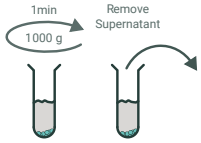


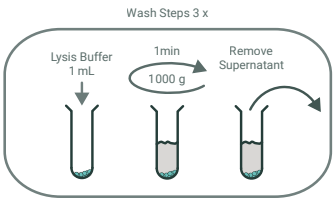
III. Bead Incubation with Supernatant

10. Resuspend equilibrated GFP-Catcher (agarose beads) gently with the cell lysate supernatant.
11. Rotate the microcentrifuge tubes for 1 h at 4 °C.
12. Centrifuge microcentrifuge tubes for 1 min at 1000 x g at 4 °C. Keep a small sample as “unbound” fraction. Carefully remove the supernatant.
13. Resuspend GFP-Catcher (agarose beads) in 1 mL Lysis Buffer.
14. Centrifuge GFP-Catcher (agarose beads) for 1 min at 1000 x g and carefully remove the supernatant.
15. Repeat wash steps twice for a total of three washes.





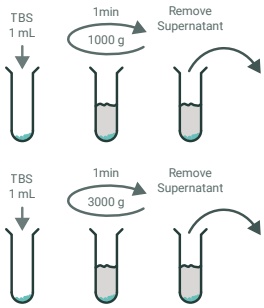




Step-by-step Protocol

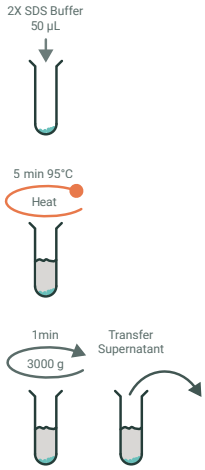
IV. Bead Washing and Solution Changes

16. Resuspend GFP-Catcher (agarose beads) gently in 1 mL TBS.
17. Centrifuge GFP-Catcher (agarose beads) for 1 min at 1000 x g and carefully remove the supernatant.
18. Resuspend GFP-Catcher (agarose beads) gently in 1 mL TBS.
19. Centrifuge GFP-Catcher (agarose beads) for 1 min at 3000 x g and carefully remove the supernatant.



V. Elution Preparation

20. Resuspend GFP-Catcher (agarose beads) resin in 50 µL 2X SDS sample buffer.
21. Heat sample (agarose beads) resin for 5 min to 95 °C.
22. Centrifuge microcentrifuge tubes for 1 min at 3000 x g and transfer the supernatant to fresh microcentrifuge tubes. Keep the pellet (agarose 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