

Protocol for ABIN7529450

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

Step-by-Step Protocol

Cell Collection & Lysis

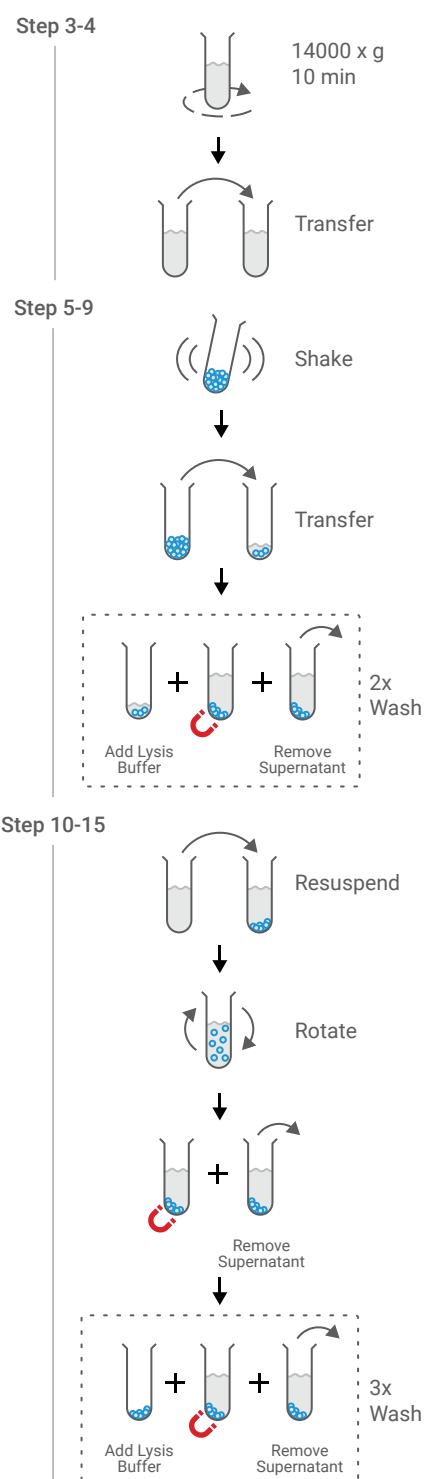
1. For mammalian cells, harvest 10^6 - 10^8 cells per sample.
2. Lyse cells according to established protocols in 0.2 to 1.5 mL volume.
Buffer recommendations:
 - 2 % Triton X-100, 1 % Tween-20, 1 % NP-40, 1 % CHAPS, 1 % Deoxycholate, 0.1 % SDS
 - 4 M NaCl, 2 M KCl, 1 M $MgCl_2$, 100 mM EDTA
 - 4 M urea
 - 10 mM DTT, 10 mM 2-Mercaptoethanol
 - RNase A, DNase I, Benzonase, protease inhibitors
3. Centrifuge cell lysates in microcentrifuge tubes for 10 minutes at 14000 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.

Bead Preparation for RFP Capture

5. Homogenize the RFP-Catcher (agarose magnetic beads) slurry gently by shaking.
6. Transfer 20 μ L 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 and carefully remove the supernatant.
9. Repeat wash steps once for a total of 2 washes.

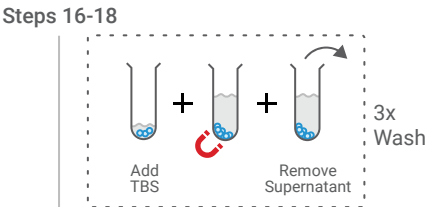
Bead Incubation with Supernatant

10. Resuspend equilibrated RFP-Catcher (agarose magnetic beads) gently with the cell lysate supernatant.
11. Rotate the microcentrifuge tubes for 1 hour at 4°C.
12. Place the tubes on a magnet stand until the fluid is clear. Keep a small sample as "unbound" fraction. Carefully remove the supernatant.
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 carefully remove the supernatant.
15. Repeat wash steps twice for a total of 3 washes.



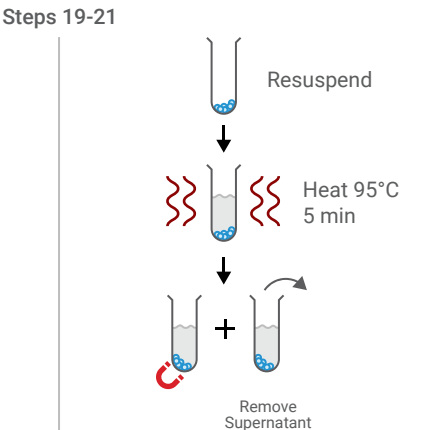
Bead Washing and Solution Changes

- 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 2 washes.



Elution Preparation

- 19. Resuspend RFP-Catcher (agarose magnetic beads) resin in 50 µL 2X SDS sample buffer.
- 20. Heat sample (agarose magnetic beads) resin for 5 minutes to 95°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.



Explore our Full Catcher Product Line

Product	Item No.
GFP-Catcher	ABIN5311508
GFP-Catcher Magnetic Beads	ABIN7272855
RFP-Catcher	ABIN5311510
RFP-Catcher Magnetic Beads	ABIN7529450
BFP-Catcher	ABIN5311512
GST-Catcher	ABIN5311506
MBP-Catcher	ABIN5311504
mNeonGreen-Catcher	ABIN7529451

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Version Mar 2024