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Protocol for ABIN7529451

mNeonGreen-Catcher High-affinity anti-mNeonGreen Single-Domain Antibody (sdAb)

Step-by-Step Protocol

Cell Collection & Lysis

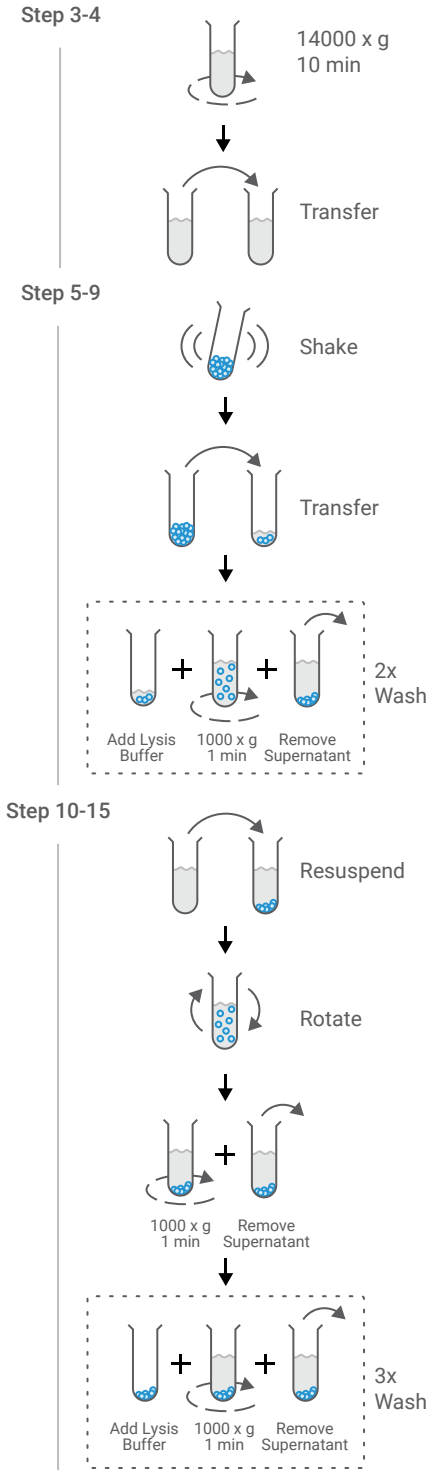
1. For mammalian cells, harvest 10^6-10^8 cells per sample.
2. Lyse cells according to established protocols in 0.2 to 1.5 mL volume. Buffer recommendations:
   - 2 % Triton X-100, 1 % Tween-20, 1 % NP-40, 1 % CHAPS, 1 % Deoxycholate, 0.1 % SDS
   - 4 M NaCl, 2 M KCl, 1 M $MgCl_2$, 100 mM EDTA
   - 4 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 mNeonGreen Capture

5. Homogenize the mNeonGreen-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 mNeonGreen-Catcher (agarose beads).
8. Centrifuge mNeonGreen-Catcher (agarose beads) for 1 minute at 1000 x g and carefully remove the supernatant.
9. Repeat wash steps once for a total of 2 washes.

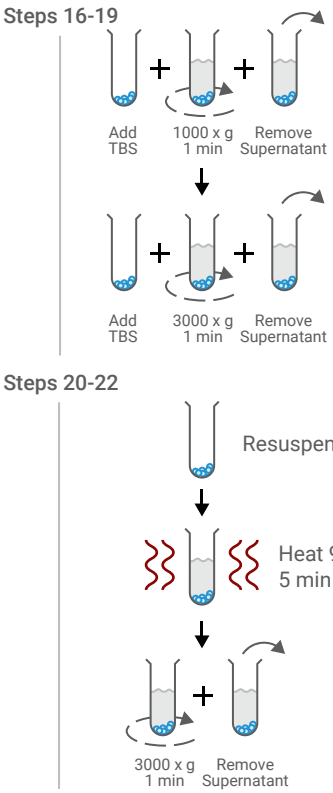
Bead Incubation with Supernatant

10. Resuspend equilibrated mNeonGreen-Catcher (agarose beads) gently with the cell lysate supernatant.
11. Rotate the microcentrifuge tubes for 1 hour at 4°C.
12. Centrifuge microcentrifuge tubes for 1 minute at 1000 x g at 4°C. Keep a small sample as “unbound” fraction. Carefully remove the supernatant.
13. Resuspend mNeonGreen-Catcher (agarose beads) in 1 mL Lysis Buffer.
14. Centrifuge mNeonGreen-Catcher (agarose beads) for 1 minute at 1000 x g and carefully remove the supernatant.
15. Repeat wash steps twice for a total of 3 washes.



Bead Washing and Solution Changes

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



Elution Preparation

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