

Protocol for ABIN5311510

## RFP-Catcher High-affinity anti-RFP Single-Domain Antibody (sdAb)

### Step-by-Step Protocol

#### Cell Collection & Lysis

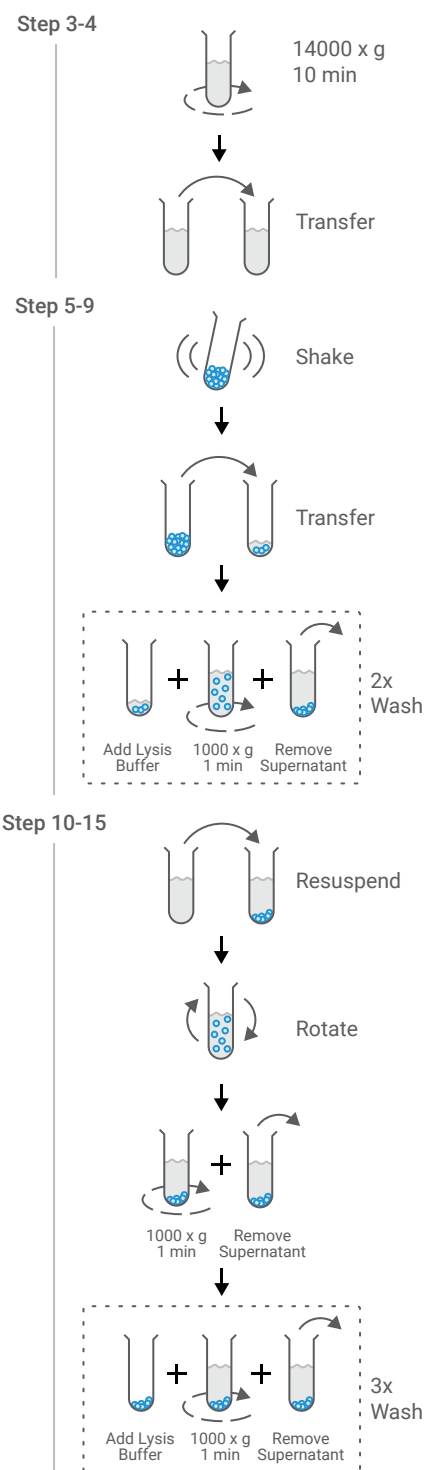
- For mammalian cells, harvest  $10^6$ - $10^8$  cells per sample.
- Lyse cells according to established protocols in 0.2 to 1.5 mL volume.  
Buffer recommendations:
  - 2 % Triton X-100, 1 % Tween-20, 1 % NP-40, 1 % CHAPS, 1 % Deoxycholate, 0.1 % SDS
  - 4 M NaCl, 2 M KCl, 1 M  $MgCl_2$ , 100 mM EDTA
  - 4 M urea
  - 10 mM DTT, 10 mM 2-Mercaptoethanol
  - RNAse A, DNase I, Benzonase, protease inhibitors
- Centrifuge cell lysates in microcentrifuge tubes for 10 minutes at 14000 x g at 4°C. Keep a small samples as "input" fraction.
- Transfer the supernatant to a fresh microcentrifuge tube for each sample and keep at 4°C.

#### Bead Preparation for RFP Capture

- Homogenize the RFP-Catcher (agarose beads) slurry gently by shaking.
- Transfer 20  $\mu$ L bead slurry to a 1.5 mL microcentrifuge tube for each sample.
- Add 1 mL Lysis Buffer to equilibrate RFP-Catcher (agarose beads).
- Centrifuge RFP-Catcher (agarose beads) for 1 minute at 1000 x g and carefully remove the supernatant.
- Repeat wash steps once for a total of 2 washes.

#### Bead Incubation with Supernatant

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

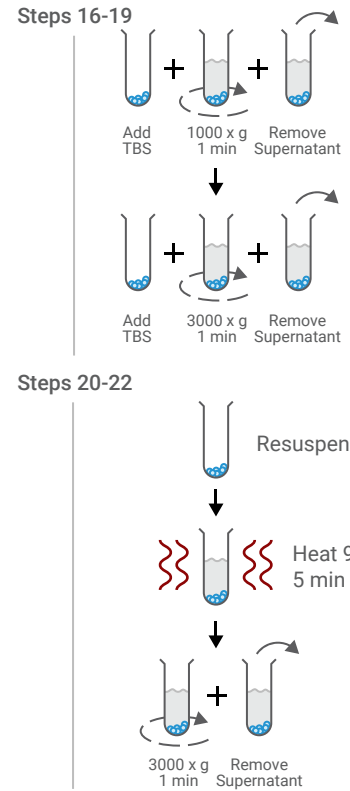


## Bead Washing and Solution Changes

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

## Elution Preparation

20. Resuspend RFP-Catcher (agarose beads) resin in 50  $\mu$ L 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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