

A. Anti-Flag resin purification of mBPI

Binding Buffer (TBS): 50 mM Tris-HCl, pH 7.5, 150 mM NaCl

Elution Buffer: 0.1 mM glycine-HCl, pH 3.5.

1. Resuspended the anti-Flag resin to form a uniform slurry and transferred 40 μ l of the slurry into a clean tube.
2. **Equilibration:** added 500 μ l Binding buffer and gently mixed the resin. Centrifuged at 6,000 \times g for 30 seconds, removed the supernatant carefully. Repeated this step two more times. Carefully removed as much supernatant as possible after each wash without disturbing the settled resin.
3. **Binding:** added 1 mL of lysate to the tube. Mixed on a tube rotator for 3 h RT. Centrifuged at 6,000 \times g for 30 seconds. Carefully removed supernatants (FT).
4. **Washing:** added 200 μ l (5 CV) of Binding buffer and mixed gently to wash the resin. Centrifuged at 6,000 \times g for 30 seconds, and removed the supernatant carefully without disturbing the resin (W). Repeated this step two more times.
5. **Elution** added 120 μ l of elution buffer into the washed resins and resuspended the resin. Incubated at RT for 5 minutes, mixed gently by tapping the tube once or twice during the incubation period. After incubation, centrifuged at 6,000 \times g for 30 seconds. Carefully transferred supernatants into a new tube containing 10 μ l 1 M Tris, pH 8.0 to neutralize the pH (E1). Repeated the elution process (E2).

B. SDS-PAGE and WB analysis

1. Run in NuPage BisTris 4-12% gel with buffer MES 15 μ l of lysate (BC), FT and E + 5 μ l LB 4x (+ BME). Incubated samples at 70 degrees for 10 min. centrifuged at 13 K for 5 min to remove insoluble material.
2. Transfer 2 parts of gel to nitrocellulose membrane and dry mb ON.
3. Wet the mb in TBS for 5 min.
4. Block for 1 h with 10 mL blocking buffer (BB).
5. Incubate for 2 h with 4 μ l of anti-Flag antibody in 10 mL diluted BB (5 mL BB+5 mL TBST + 75 μ l Tween-20 20%) dilution (1:2500) and 3 μ l anti-BPI in 6 mL diluted BB (3 mL BB+3 mL TBST + 45 μ l Tween-20 20%)
6. Wash TBST 3X, 5 min.
7. Incubate for 1 h with 1 μ l of anti-rabbit IRDye in 10 mL diluted BB.
8. Wash TBST 3X , 5 min.
9. Wash with TBS