High-throughput Hisx6-tagged protein purification

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Introduction

We have developed a high-throughput protein purification protocol that allows us to purify ~4,000 proteins within 10 hours from prepared culture. By combining steps of cell lysis and protein capture on affinity resins in sealed filter plates, we reduced the number of pipetting steps and thus, human errors.

Equipment

Q-Fill2

Procedure

1. Re-suspend cell pellets at 4 °C in 80 µL of lysis buffer, containing 50 mM NaH_2PO_4 at pH 8 with 300 mM NaCl, 20 mM imidazole, CelLytic B (Sigma), Lysozyme (1 mg/mL), Benzonase (50 units/mL), proteinase inhibitor cocktail (Sigma), and PMSF (1 mM/mL).
2. Wash Ni-NTA Superflow (QIAGEN) twice with water and twice with 50 mM NaH_2PO_4 at pH 8 with 300 mM NaCl, 20 mM imidazole using centrifugation.
3. Add 25 µL pre-washed Ni-NTA Superflow (QIAGEN) to each well
4. Transfer the mixtures into bottom-sealed filter plates (Multiscreen Nylon Mesh)
5. Seal the filter plates
6. Incubated for 1.5 hr at 4 °C with vigorously shaking
7. Remove seals
8. Wash the resin-protein complexes 3 times with 250 µL/well of wash buffer I (50 mM NaH_2PO_4 with 300 mM NaCl, 10% glycerol, 20 mM imidazole, 0.01% Triton X-100, at pH 8) and 3 times with Wash buffer II (50 mM NaH_2PO_4 with 150 mM NaCl, 25% glycerol, 20 mM imidazole, 0.01% Triton X-100, at pH 8) using a Q-fill2 (Gentix, UK).
9. Spin the filter plates
10. Add 25 µL of elution buffer (50 mM NaH_2PO_4/150 mM NaCl/25% glycerol/250 mM imidazole/0.01% Triton X-100, pH 7.5)
11. Incubate 15 min at 4 °C

12. Elute proteins into 96-well receiver plates by centrifugation.

13. Repeat 10-12

14. Store the proteins in 96-well plates at -80 °C

Timing

10 hours

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A proteome chip approach reveals new DNA damage recognition activities in Escherichia coli

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