

His GraviTrap による精製後の SDS-PAGE

Method:

Equilibration: 10 ml binding buffer
Sample application: 20 ml unclarified *E. coli* BL21 lysate containing 14.8 mg GFP-(His)₆
or
10 ml unclarified *E. coli* DH5α lysate containing 7.4 mg GFP-(His)₆
or
10 ml clarified *E. coli* BL21 lysate containing 7.4 mg GFP-(His)₆

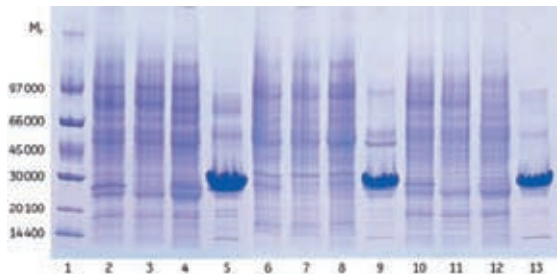
Wash: 10 ml binding buffer

Elution: 3 ml elution buffer

Buffers:

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 45 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4



1. LMW markers
2. Unclarified sample (20 ml), start material (diluted 1:10)
3. Unclarified sample (20 ml), flowthrough (diluted 1:10)
4. Unclarified sample (20 ml), wash
5. Unclarified sample (20 ml), eluate
6. Unclarified sample (10 ml), start material (diluted 1:10)
7. Unclarified sample (10 ml), flowthrough (diluted 1:10)
8. Unclarified sample (10 ml), wash
9. Unclarified sample (10 ml), eluate
10. Clarified sample (10 ml), start material (diluted 1:10)
11. Clarified sample (10 ml), flowthrough (diluted 1:10)
12. Clarified sample (10 ml), wash
13. Clarified sample (10 ml), eluate

遠心分離やフィルトレーションしたサンプル (clarified)、していないサンプル (unclarified) で結果に差は見られませんでした。