Protocol 2-1: Protein Isolation for prokaryotes

General Protocol

1) Bacteria of positive clone are grown in sterilized medium containing 1% anti-biotics at 37 °C.
2) At OD600 = 0.8, or overnight, cells are induced by addition of IPTG and left at 25 °C overnight.
3) The suspension is then centrifuged (30 min, 4500 rpm, 6 °C) and the pellet resuspended in lysis buffer (20 mM imidazol, 0.25M NaCl) and the cell suspension sonicated two cycles, using an ultrasonic processor. For each cycle, the working time/ pausing time is 4s/ 6s.
4) The cells are pelleted by centrifugation at 12000rpm for 50 min at 10 °C, using a high-speed centrifuge.
5) The supernatant is loaded on a Ni-NTA column, washed with lysis buffer and eluted with Buffer B (400 mM imidazol).
6) Freeze the fractions at the temperature of -20 °C, for other detections.

Reference

1) Sambrook J, Maniatis T, Fritsch EF. Molecular Cloning: a Laboratory Manual. cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 3rd ed., 2001.
2) Robert F. Weaver. Molecular Biology, McGrawHill, 4th edition, 2007