

GST protein purification

Typical GST fusion proteins can be produced from 1 litre of *E.coli* BL21 cells grown in a rich medium. A standard induction protocol entails shifting log-phase cultures ($A_{600} = \sim 0.6$) from 37°C to room temperature and adding IPTG to a final concentration of 100 μ M.

After 5 h at room temperature with constant vigorous shaking, recover the bacteria by centrifugation at 5,000 rpm (GS3 rotor) at 4°C for 15 min and store at -80°C until ready to proceed with the purification.

Lyse cell pellets in 20 ml Lysis Buffer for 20 min at room temperature, briefly sonicate and remove the insoluble material by centrifugation at 42,000 rpm (Ti45 rotor) at 4°C for 25 min.

Mix the clarified lysate on a tumbler at 4°C for 4 h (or 1 h at room temperature) with glutathione-Sepharose 4B (AP Biotech) preequilibrated with 20 mM Tris pH 7.5, containing 0.1 % Triton X-100.

Wash four times with 20 mM Tris pH 7.5, 0.1 % Triton X-100, followed by a single wash with detergent-free 50 mM Tris pH 8.0.

Elute the GST-fusion proteins with 10 mM reduced glutathione in 50 mM Tris pH 8.0. Dialyse eluted proteins overnight against PBS before use in pull-down experiments.

Lysis Buffer

20 mM Tris pH 7.5
0.1 % Triton X-100
1 mg/ml lysozyme
1 μ g/ml DNase