Protocol for purification of streptavidin fusion protein

Transformants of E.coli strain BL21 are grown overnight at 37℃ with shaking in LB medium supplemented with suitable concentration of antibiotic. The culture is diluted 50-fold into fresh LB medium with antibiotic and grown at 37℃ for 8h to 12h. Then the culture is diluted 100-fold into fresh LB medium with antibiotic and grown in a shaking incubator at 37℃. When the OD600 of the culture reaches 0.6 to 0.8, IPTG is added to a final concentration of 200μM. The induced cells are incubated 12h at 37℃ with shaking.

Cells are harvested by centrifugation at 6000rpm for 10min at 4℃. The cells are resuspended in buffer A (20mM Tris, 500mM NaCl, pH 8.0) and disrupted by ultrasonic cell disrupter. The crude cell lysate is separated into soluble and insoluble fractions by centrifugation at 15000rpm for 50min at 4℃. The insoluble fraction is washed twice by washing buffer (0.5% Triton-100, 50mM Tris, 300mM NaCl, 10mM EDTA, 10mM DTT) to remove cell fragment.

The insoluble fraction is resuspended in resuspension buffer (50mM Tris, 100mM NaCl, 10mM EDTA, 10mM DTT, pH 8.0) and disrupted by ultrasonic cell disrupter. The inclusion bodies are harvested by centrifugation at 12000rpm for 10min at 4℃.

Per 30mg inclusion body is dissolved in 1mL dissolution buffer (6M guanidine-HCl, 10% glycerol, 50mM Tris, 100mM NaCl, 10mM EDTA, 10mM DTT), followed by refolding via dilution into refolding buffer (100mM Tris, 400mM L-Arg-HCl, 2mM EDTA, 5mM GSH, 0.5mM GSSG, pH 8.0).

The refolding protein is concentrated to a proper volume and dilute to protein buffer (50mM Tris, 100mM NaCl). Protein purity is checked by SDS-PAGE, and the resulting protein is quantified by spectrophotometry.