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PDI variants expression and purification protocol

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1 Human PDI was clone in the pT7-FLAG-SBP-1vector (sigma), designed for expression in E.coli BL21 (DE3) T1 cells (sigma).

2 Inoculate 10-ml Transformed E.coli BL21 (DE3) T1 cells on the medium containing ampicillin.

3 Grow the culture O/N at 37degree.

4 Transfer 10ml cell culture to 1Liter LB medium (100µg/ml Amp) and shake at 200rpm, 37 degrees.

5 When OD600 reaches 0.4-0.6, add IPTG to a final concentration of 1mM.

6 Keep the induction temperatures at 22-25 degrees for 13hours.

7 Harvest cells at **7000 rpm, 4°C, 00:10:00** . And this can be store at -20degrees until needed

8 Suspend harvest cells in 30ml buffer containing 1U/ml Nuclease, 50 µg/mL DNase, 200 µg/mL Lysozyme,1mM PMSF, 2mM DTT, 2mM EDTA and 1tablet Protease inhibitor cocktail.

Alternatively, B-PER reagent can be used to solubilize and lyse the cells.

9 After 30min incubation, sonicate the mixture 10 sec at intensity 10W, 10 times. This will reduce the viscosity.

10 After centrifugation at 11000rpm for 30min, load the soluble supernatant to High CapacityStreptavidin Agarose column pre-equilibrium with wash buffer consist of 20mMTris HCl pH 8.0, 300mM NaCl, 2mM EDTA, 2mM DTT.

11 The target protein was eluted with 2mM Biotin in the wash buffer followed extensively wash.

12 Dialyze the eluted protein subsequently exhaustively against 1xPBS, pH7.4. It is recommended to have three times dialysis with a minimum of 2-4 hs each.

13 Determine the protein concentration using the mass extinction coefficient of 8.62 at 280 nm.

14 Check the purity of recombinant PDIs by SDS-PAGE using a 7.5% gel.

15 Monitor the enzymatic activities of the PDI variants using the insulin reductase assay.

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