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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.