BMC	Standard Operation Procedure Preparing a selenomethionyl-labeled protein in Ecoli cells

1. Aim:

Selenomethionine labeling of Escherichia coli-expressed recombinant protein

2. Applicability:

Selenium can be used for phase determination in multi-wavelength anomalous diffraction (MAD) method. Se-Met can often replace methionine residues in a protein without affecting the protein's properties, therefore producing a protein advantageous for crystal structure solving. Also, the X-ray absorption edge of selenium is easily accessible by synchrotron radiation, making a Se-Met crystal ideal for collecting anomalous X-ray diffraction data.

3. Responsibilities:

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4. Required materials:

WARNING! SeMet is TOXIC. The culture supernatant should be collected for disposal as hazardous waste.

Antibiotics (Calbiochem, DE)

M9 media (minimal media) (Sigma-Aldrich, USA)

MEM Amino Acids Solution (50X) (Gibco, USA)

Glucose (sterile) and Thiamine Solution (Sigma-Aldrich, USA)

Tryptone, yeast extract, and agar (Difco, USA)

E. coli BL21(DE3) competent cells (ABO, Poland)

Plasmid Mini Kit (Promega, USA)

HisTrap 1ml or 5 ml (GE Healthcare, USA)

Ball mill (PM100; RETSCH GmbH, Germany)

Isopropyl-β-D-thiogalactopyranoside (IPTG) (Sigma-Aldrich, USA)

β-mercaptoethanol (βME) (Sigma-Aldrich, USA)

BlueStar PLUS and BlueEasy Prestained Protein Ladders (ABO, Poland)

Vivaspin® concentrators (Sigma-Aldrich, USA) or Amicon® Ultra concentrators (Merck, Germany)

5. Procedure:

A. Preparation of bacterial E.coli culture, media and stock solutions

Day 1-2: Grow the overnight culture in minimal media

Prepare a 2 mL day culture consisting of 2 mL LB media, 2 uL antibiotics (1000x conc.), and a single E.coli colony. Grow at 37 °C all day. Prepare M9 stock media from purchased powder. Dilute and autoclave before use. Prepare amino acid 50x Stock. Prepare a 150 mL overnight culture consisting of 150 mL LB, 150 uL antibiotics (1000x conc.), and 150 uL of day culture. Grow at 37°C overnight.

Day 3:

Inoculate minimal media with overnight culture

To each liter M9 (1x conc.) add:

- 10 mL 20 % Glucose (sterile filtered or autoclaved)
- 2 mL 1 M $MgSO_4$ (sterile filtered)
- 0.05 mL 2 M $CaCl_2$ (sterile filtered)
- 0.1 mL 0.5% (w/v) thiamine solution (sterile filtered)
- 1 mL antibiotics (1000x conc.)
- 20 mL amino acid 50x Stock (If precipitate is seen, heat to 60-70 °C and shake)

Inoculate M9 with 50 mL overnight culture from Day 2 and grow until an OD600 = 0.5 – 0.6 (~2.0 – 2.5 hours).

Add amino acid mix to inhibit methionine synthesis and to supply selenomethionine

Add 100 mg threonine, lysine hydrochloride, phenylalanine to the culture. Add 50 mg leucine, isoleucine, valine to the culture (all as solid powders). Add 120 mg DL-Se-Met or 60 mg L-Se-Met to the culture (as a solid powder). Continue to grow the culture for 15 minutes.

Induction, expression and harvesting

Induce with 1 mL 1 M IPTG (final concentration = 1 mM) and let it grow for about 6-8 hours (whatever is optimal for the protein of interest). Collect cells as usual and proceed to purification steps.

Note: Use all amino acids except Gly, Ala, Pro, Asn, Cys, and Met at a concentration of 2 mg/ml. To help in dissolving the amino acids, autoclave for 10 minutes.

B. Purification of Se-labeled protein by Ni-agarose beads (or HisTrap column)

Day 4-5

Mix 5 ml of the nickel-chelate slurry thoroughly until homogeneous suspension is visible. Transfer the gel suspension into an appropriate column with inner diameter of 1.0 to 1.5 cm.

After column preparation equilibrate the column with selected Binding buffer by washing with 5-10 column volumes. Recommended flow rates are 1-2 ml/min/cm2.

Apply the sample to column at a rate between 0.1 ml/min to 0.5 ml/min using a syringe or a pump. A partial displacement of chelated nickel ions is often noted as the protein is adsorbed (the blue color is changed to off-white color). The total volume of the sample applied is not critical in most cases. Save the flow through for SDS-PAGE analysis.

Wash with 5-10 column volumes of Binding buffer. To increase the purity of eluted protein a wash with Binding buffer containing 5-50 mM imidazole is often effective. If the protein of interest is present in lysate supernatants but has not bound well to the column the imidazole concentration added to lysate should be restricted to 1 mM final concentration in order to improve binding.

Elute with selected Elution buffer (containing Imidazole), at flow rates of 1-2 ml/min/cm^2. Two to five column volumes are usually needed for elution of the His tagged protein. A gradient type of elution where imidiazole concentration ranges between 0 and 500 mM may be used to improve the separation of the target protein from contaminants. A linear gradient with 10-20 column volumes is recommended. Typically, imidazole concentrations of 100 mM to 200 mM are sufficient to elute most His tagged proteins.

Note: 500 mM imidazole has A280 ~0.5. Use the Elution buffer as blank when quantitation of the target protein in eluted fractions.

C. Gel filtration of finaly purified Se-Met-labeled protein

Day 6

In the final step of purification process, the protein incorporated with selenomethionine was loaded onto Superdex 200 column with an internal diameter of 20 mm and length of 30 cm connected to AKTA Pure system (GE Healthcare). Gel filtration was performed using HEPES (pH 7.5) at a flow rate of 0.5 mL/min. The protein was eluted and the process was monitored by measuring UV absorbance at 280 nm. The collected fractions (0.5 mL) were analyzed by SDS-PAGE and analytical SEC. The selected fractions were concentrated, and the final protein concentration was determined using NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). The yield of purified protein labeled with selenomethione obtained from isolation from E. coli cells was usually between 0.5 mg/L – 2.0 mg/L.

Note: Se-Met protein will show slightly larger MW than the native protein in mass spectrum and SDS-PAGE. Se-Met protein may behave slightly differently from the native protein in purifications and crystallization.

The development of this SOP was supported by the programme Interreg V – A Slovakia - Austria, project CAPSID.