Expression of selenomethionine substituted proteins in non-methionine auxotrophic *E. coli*

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Modified from:
Van Duyne GD, Standaert RF, Karplus PA, Schreiber SL, Clardy J. Atomic structures of the human immunophilin FKBP-12 complexes with FK506 and rapamycin. J Mol Biol. 1993 Jan 5;229(1):105-24.

The protein is expressed in any *E. coli* strain, with no need for methionine synthesis deficient strains. Feed back inhibition of methionine biosynthesis is facilitated by adding amino acids prior to induction. The following protocol is for 10l culture (version 11 June 2003).

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1. freshly transformed plate over night

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2. 5ml pre-culture in rich medium (2xTY, LB) starting in the morning

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3. 300ml pre-culture in minimal medium (see below) over night, 1:1000 inoculum (300μl)

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4. 10x1l culture in minimal medium (see below) starting in the morning, 1:100 inoculum (10ml)

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5. at desired OD_{600} for induction amino acids are added as *solids*. Mix *very thoroughly with a spatula:*
Feed-back inhibition amino acids mix
1.0g of lysine, threonine, phenylalanine
0.5g of leucine, isoleucine, valine
0.5g of L(+) selenomethionine (ACROS Organics 259960025)
The mix is divided into 10 0.5g portions and the amino acids are added to the culture flasks

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6. after 15min, expression is induced

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7. expression temperature and duration as desired

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8. purification: all buffers *must* contain either 5mM β-mercapto ethanol (nickel columns) or 5mM DTT (all others). Add reducing agents immediately before use.

Minimal medium (per litre):
1l M9 medium
2ml 1M $MgSO_4$ (2mM)
20ml 20% glucose (0.4%)
antibiotic(s)
1ml vitamins 1000x (see below)
10ml trace elements 100x (see below)

1l 100x trace elements
in this order:
5g EDTA
0.8g $FeCl_3$
0.05g $ZnCl_2$
0.01g $CuCl_2$
0.01g $CoCl_2$
0.01g H_3BO_3
1.6g $MnCl_2$
some Ni_2SO_4
some molybdic acid
dissolve, bring pH to 7.0 with NaOH (some precipitation)
sterile filtered, kept chilled

500ml 1000x vitamins
0.5g riboflavin
0.5g niacinamide
0.5g pyridoxine monohydrate
0.5g thiamine
sterile filtered, kept chilled