

Supplementary Table 2 Effect of media composition on the contents of ThTP and AThTP in *E. coli*

Incubation medium	ThTP (pmol/mg of protein)	AThTP (pmol/mg of protein)
Minimal M9 medium	n. d.	111 ± 11
M9 + D-glucose (10 mM)	240 ± 40	n. d.
Nitrogen-free minimal medium (a)	n. d.	79 ± 13
Nitrogen-free minimal medium + D-glucose (10 mM)	326 ± 60	n. d.
Phosphate-free minimal medium (b)	n. d.	120 ± 15
Phosphate-free minimal medium + D-glucose (10 mM)	121 ± 2	n. d.
M9 + pyruvate (10 mM)	90 ± 39	n. d.
M9 + L-lactate (10 mM)	67 ± 10	5 ± 1
M9 + L-malate (10 mM)	n. d.	n. d.
M9 + amino acids (c)	15 ± 4	n. d.

The bacteria (BL-21) were grown overnight in LB medium, suspended in minimal medium (M9 or modified as indicated) and incubated 1 h at 37 °C, 250 rpm. After incubation, the bacteria were sedimented (5 min, 10,000 x g) and the pellets were suspended in 12% (w/v) trichloroacetic acid and centrifuged. The supernatant was extracted with diethyl ether and the thiamine compounds were determined by HPLC after derivatization to thiochrome derivatives. The results are expressed as mean ± SD for 3 – 6 experiments.

n. d.: not detected (< 5 pmol/mg for ThTP and < 2.5 pmol/mg for AThTP)

M9 medium contained Na₂HPO₄, 6 g/l; KH₂PO₄, 3 g/l; NaCl, 0.5 g/l; NH₄Cl, 1 g/l; CaCl₂, 3 mg/l; MgSO₄, 1 mM, pH 7.0

(a) The nitrogen-free medium had the same composition as M9 medium but NH₄Cl was omitted and the NaCl concentration was 1.5 g/l

(b) The phosphate-free minimal medium contained HEPES-Na 20 mM, pH 7.0 ; NaCl, 52 mM ; KCl, 11 mM ; NH₄Cl, 1 g/l ; CaCl₂, 3 mg/l ; MgSO₄, 1 mM.

(c) The amino acid mixture contained all 20 amino acids, each at 0.5 mM except for tyrosine (0.05 mM) and tryptophan (0.1 mM).