Preparing Energy solution for cell free Tx-TL reactions

Andres Medina

Abstract

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Protocol

Preparing stock solutions

Step 1.

DTT, 1 M: Prepare 2.31 g DTT and water to 15 ml. Filter sterilize (0.22 µM), aliquot to 1 ml tubes, store at -20 °C for later use.

S30A buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, 50 ml Tris at 2M, acetic acid (to pH 7.7), and water to 2 L. Autoclave, store at 4 °C, add 4 ml 1 M DTT before use.

S30B buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, Tris at 2 M (to pH 8.2), and water to 2 L. Autoclave, store at 4 °C, add 2 ml 1 M DTT before use.

HEPES: Prepare 1.91 g HEPES (MW 238.21), KOH (to pH 8), and water to 4 ml.

tRNA: Prepare 30 mg of tRNA and water to 600 µl.

CoA: Prepare 30 mg of CoA (MW 767.53) and water to 600 µl.

NAD: Add 34.83 mg of NAD (MW 663.43), Tris at 2 M (to pH 7.5-8), and water to 300 µl. (Add 27 µl of Tris at 2 M to bring the solution to pH 7.5-8).

cAMP: Add 42.80 mg of cAMP (MW 329.22), Tris at 2 M (to pH 8), and water to 200 µl. (Add 73 µl of

Tris at 2 M to bring the solution to pH 8).

Folinic Acid (33.9 mM): To 20 mg of solid folinic acid calcium salt (MW 511.5), add 1.15 ml water.

Spermidine: Prepare 23.55 µl of spermidine (MW 145.25) and water to 150 µl. Prepare at room temperature after melting briefly at 37 °C.

3-PGA: Add 1.03 g of 3-PGA (MW 230.02), Tris at 2 M (to pH 7.5), and water to 3.2 ml. (Add 1.73 ml of Tris at 2 M to bring the solution to pH 7.5).

Nucleotide Mix: Add 145 mg of ATP dipotassium salt dihydrate (MW 619.4), 133 mg of GTP disodium salt (MW 567.14), 79.4 mg of CTP disodium salt dihydrate (MW 563.16), 82.6 mg of UTP

trisodium salt dihydrate (MW 586.12), KOH at 15% dilution (to pH 7.5), and water to 1.5 ml. (Add 353 µl of KOH at 15% dilution to bring the solution to pH 7.5).

Preparing stock solutions

Step 2.

STOCK	Volumen to take (µL)
HEPES pH 8	500
Nucleotide mix	198
NAD	38.33333333
CO-A	80
cAMP	23
Folinic acid	40
spermidine	17
Maltodextrine	1000
TOTAL	1896.333333