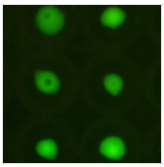

Version 3
Oct 17, 2018
Working

Step 2: Preparing amino acid, polyphosphates, and maltodextrin-based energy solutions for cell-free reactions Version 3

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dx.doi.org/10.17504/protocols.io.urzev76
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PROTOCOL STATUS

Working
We use this protocol in our group and it is working

STEPS MATERIALS

NAME	CATALOG #	VENDOR
L-aminoacids	LAA21–1KT	Sigma Aldrich
L-Proline	81709–25G	Sigma Aldrich
L-Cysteine	30089	Sigma Aldrich
L-histidine	53319–25G	Contributed by users
L-lysine	L5501–5G	Contributed by users
DTT	D0632	Sigma Aldrich
CoA	C4282	Sigma Aldrich
Folinic Acid	F7878	Sigma Aldrich
Spermidine	85558	Sigma Aldrich
NAD (β-Nicotinamide adenine dinucleotide hydrate)	N6522	Sigma Aldrich
HEPES	H6147	Sigma Aldrich
ATP (Adenosine 5′-triphosphate dipotassium salt hydrate)	A8937	Sigma Aldrich
GTP (Guanosine 5′-triphosphate sodium salt hydrate)	10106399001 ROCHE	Sigma Aldrich
UTP (Uridine 5′-triphosphate trisodium salt dihydrate)	94370	Sigma Aldrich
Maltodextrin (4.0-7.0 dextrose equivalent)	419672	Sigma Aldrich
sodium hexametaphosphate	305553	Sigma Aldrich

Prepare individual amino acid stock solutions

1 Dissolve the given amount of each amino acid with 1 ml of 5M KOH in a 3 ml screw-cap tube according to the following list.

Aminoacid	Amount of powder needed
Alanine	445.45 mg

Arginine	644.6502 mg
Asparagine	660.6 mg
Aspartic acid	665.5 mg
Cysteine	370.7496 mg
Glutamic acid	735.65 mg
Glutamine	447.219 mg
Glycine	375.35 mg
Histidine	775.75 mg
Isoleucine	655.9 mg
Leucine	401.4108 mg
Lysine	447.3414 mg
Methionine	456.5826 mg
Phenylalanine	330.38 mg
Proline	575.65 mg
Serine	529.5 mg
Threonine	595.6 mg
Tryptophan	408.46 mg
Tyrosine	554.4414 mg
Valine	358.479 mg

Vortex to dissolve. Each solution can be stored at -20 °C.

REAGENT
L-aminoacids
by Sigma Aldrich
Catalog #: LAA21−1KT

REAGENT
L-Proline
by Sigma Aldrich
Catalog #: 81709−25G

REAGENT
L-Cysteine
by Sigma Aldrich
Catalog #: 30089

REAGENT
L-histidine
Contributed by users
Catalog #: 53319−25G

REAGENT
L-lysine
Contributed by users
Catalog #: L5501−5G

Prepare amino acid mix solution (~17 nM each)

2 In a 50 mL Falcon tube add the following volumes of the individual amino acid stock solutions

Aminoacid Stock solution in 5M KOH	Volume needed

Alanine	136 ul
Arginine	222 ul
Asparagine	136 ul
Aspartic acid	136 ul
Cysteine	222 ul
Glutamic acid	136 ul
Glutamine	222 ul
Glycine	136 ul
Histidine	136 ul
Isoleucine	136 ul
Leucine	222 ul
Lysine	222 ul
Methionine	222 ul
Phenylalanine	340 ul
Proline	136 ul
Serine	136 ul
Threonine	136 ul
Tryptophan	340 ul
Tyrosine	222 ul
Valine	222 ul
TOTAL	**3816 µL**

Prepare amino acid mix solution (~14 mM each)

3 Add 35.084 µL of sterile water and 1.100 µl of Acetic acid (glacial) to the amino acid mix. Vortex well and aliquot in 2.0 ml Eppendorf tubes. Aliquots can be stored at -80°C

Prepare maltodextrin-based energy solution

4 Prepare the following stock solutions:

1M DTT: Put 2.31 g DTT in a 15 ml Falcon tube and fill with water to 15 ml. Sterilize using a 0.22 µM filter. Aliquot in 1.5 ml Eppendorf tubes. Store at -20 °C for later use.
2M HEPES pH 8: Weight 19.1 g HEPES (MW 238.21). Dissolve with 30ml water. Adjust pH to 8.0 with KOH. Fill with water to 40 ml.
5 mg/ml tRNA solution: Put 30 mg of tRNA in a 1.5 ml Eppendorf tube. Fill with water to 600 µl.
CoA stock solution*:* Put 30 mg of CoA (MW 767.53) in a 1.5 ml Eppendorf tube and fill with water to 600 µl.
38.3 mM NAD solution*:* Put 34.83 mg of NAD (MW 663.43) in a 1.5 ml Eppendorf tube, add 27 µl of Tris at 2 M (to bring the solution to pH 8.0). Finally, fill with water to 300 µl.
23 mM cAMP solution*:* Put 42.80 mg of cAMP (MW 329.22) in a 1.5 ml Eppendorf tube, add 73 µl of Tris at 2 M (to bring the solution to pH 8.0). Finally, fill with water to 200 µl.
40 mM Folinic acid solution*:* To 20 mg of solid folinic acid calcium salt (MW 511.5), add 1.15 ml of water.
Spermidine stock solution*:* Briefly warm spermidine (two minutes at 37°C) in order to melt it. Then, put 23.55 µl of spermidine (MW 145.25) into a 1.5 Eppendorf tube and fill with water to 150 µl.
240 mg/ml maltodextrin solution*:* Put 2.4 g of maltodextrin in a 15 ml Falcon tube. Dissolve, and fill with water to 10 ml.
Nucleotide Mix solution*:* Put 145 mg of ATP dipotassium salt dihydrate (MW 619.4), 133 mg of GTP disodium salt dihydrate (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) in a 1.5 Eppendorf tube. Add 353 µl of KOH (15 w/v %) .Finally, fill with water to 1.5 ml.

REAGENT
DTT
by Sigma Aldrich
Catalog #: D0632

REAGENT
CoA
by Sigma Aldrich
Catalog #: C4282

REAGENT
Folinic Acid
by Sigma Aldrich
Catalog #: F7878

REAGENT
Spermidine
by Sigma Aldrich
Catalog #: 85558

REAGENT
NAD (β-Nicotinamide adenine dinucleotide hydrate)
by Sigma Aldrich
Catalog #: N6522

REAGENT
HEPES
by Sigma Aldrich
Catalog #: H6147

REAGENT
ATP (Adenosine 5′-triphosphate dipotassium salt hydrate)
by Sigma Aldrich
Catalog #: A8937

REAGENT
GTP (Guanosine 5′-triphosphate sodium salt hydrate)
by Sigma Aldrich
Catalog #: 10106399001 ROCHE

REAGENT
UTP (Uridine 5′-triphosphate trisodium salt dihydrate)
by Sigma Aldrich
Catalog #: 94370

REAGENT
Maltodextrin (4.0-7.0 dextrose equivalent)
by Sigma Aldrich
Catalog #: 419672

5 In a 15 mL Falcon tube add the following volumes of the stock solutions prepared before:

Stock solution	Volume needed
HEPES pH 8	1000 µl
Nucleotide mix	396 µl
tRNA solution	160 µl
NAD	76.6 µl
CO-A	160 µl
cAMP	46 µl
Folinic acid	80 µl
spermidine	34 µl
Maltodextrin	2000 µl
TOTAL	**3952.6 µl**

6 Dissolve well the maltodextrin-based energy solution using vortex. Aliquot in 2 ml Eppendorf tubes and store at -80°C prior to use.

Prepare hexametaphophate solution

7 Weight 0.15 g of sodium hexametaphosphate and put it into a 15 ml Falcon tube. Dissolve well and fill with water to 5 ml. Put the solution in a boiling water bath for 5 minutes. Kepp in the solution in the lab bench until it reaches room temperature. Aliquot in 1.5 ml Eppendorf tubes and keep at -80°C before use.

REAGENT
sodium hexametaphosphate
by Sigma Aldrich
Catalog #: 305553