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Step 2: Preparing amino acid, polyphosphates, and maltodextrin-based energy solutions for cell-free reactions V.1

In 2 collections

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MATERIALS

STEP MATERIALS

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

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⊗	Spermidine	Merck MilliporeSigma (Sigma-Aldrich) Catalog #85558
⊗	NAD (β -Nicotinamide adenine dinucleotide hydrate)	Merck MilliporeSigma (Sigma-Aldrich) Catalog #N6522
⊗	HEPES	Merck MilliporeSigma (Sigma-Aldrich) Catalog #H6147
⊗	ATP (Adenosine 5'-triphosphate dipotassium salt hydrate)	Merck MilliporeSigma (Sigma-Aldrich) Catalog #A8937
⊗	GTP (Guanosine 5'-triphosphate sodium salt hydrate)	Merck MilliporeSigma (Sigma-Aldrich) Catalog #10106399001 ROCHE
⊗	UTP (Uridine 5'-triphosphate trisodium salt dihydrate)	Merck MilliporeSigma (Sigma-Aldrich) Catalog #94370
⊗	Maltodextrin (4.0-7.0 dextrose equivalent)	Merck MilliporeSigma (Sigma-Aldrich) Catalog #419672
⊗	sodium hexametaphosphate	Merck MilliporeSigma (Sigma-Aldrich) Catalog #305553

Prepare individual amino acid stock solutions

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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.

Amino acid	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.

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☒ L-Cysteine **Merck MilliporeSigma (Sigma-Aldrich) Catalog #30089**

☒ L-histidine **Contributed by users Catalog #53319-25G**

☒ 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

Amino acid Stock solution in 5M KOH	Volume needed
Alanine	136 μ l
Arginine	222 μ l
Asparagine	136 μ l
Aspartic acid	136 μ l
Cysteine	222 μ l
Glutamic acid	136 μ l

Glutamine	222ul
Glycine	136ul
Histidine	136ul
Isoleucine	136ul
Leucine	222ul
Lysine	222ul
Methionine	222ul
Phenylalanine	340ul
Proline	136ul
Serine	136ul

Threonine	136 μ l
Tryptophan	340 μ l
Tyrosine	222 μ l
Valine	222 μ l
TOTAL	3816 μL

- 3 Add 35.084 μ L of sterile water and 1.100 μ l 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 μ M filter. Aliquot in 1.5 ml Eppendorf tubes. Store at -20 °C for later use.

5.3 M 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.

⊗ DTT Merck MilliporeSigma (Sigma-Aldrich) Catalog #D0632

⊗ CoA Merck MilliporeSigma (Sigma-Aldrich) Catalog #C4282

⊗ Folinic Acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #F7878

⊗ Spermidine Merck MilliporeSigma (Sigma-Aldrich) Catalog #85558

⊗ NAD (β-Nicotinamide adenine dinucleotide hydrate) Merck MilliporeSigma (Sigma-Aldrich) Catalog #N6522

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⊗ UTP (Uridine 5'-triphosphate trisodium salt dihydrate) Merck MilliporeSigma (Sigma-Aldrich) Catalog #94370

⊗ Maltodextrin (4.0-7.0 dextrose equivalent) Merck MilliporeSigma (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 μ l
Nucleotide mix	396 μ l
NAD	76.6 μ l
COA	160 μ l
CAMP	46 μ l
Folic acid	80 μ l
spermidine	34 μ l
Malto dextrin	2000 μ l

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- 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 hexametaphosphate 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.

sodium hexametaphosphate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #305553**