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

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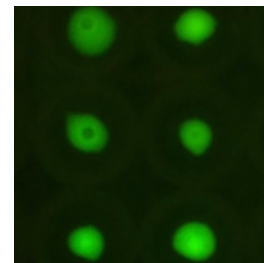
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**Protocol status:** Working

**Created:** June 12, 2017

**Last Modified:** March 28, 2018

**Protocol Integer ID:** 6311



## 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 **Catalog #53319-25G**

✕ L-lysine **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 ( $\beta$ -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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## 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.

<b>Aminoacid</b>	<b>Amount of powder needed</b>
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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## 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
<b>TOTAL</b>	<b>3816 µL</b>

- 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  $\mu$ 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  $\mu$ l.

**CoA stock solution:** Put 30 mg of CoA (MW 767.53) in a 1.5 ml Eppendorf tube and fill with water to 600  $\mu$ l.

**38.3 mM NAD solution:** Put 34.83 mg of NAD (MW 663.43) in a 1.5 ml Eppendorf tube, add 27  $\mu$ l of Tris at 2 M (to bring the solution to pH 8.0). Finally, fill with water to 300  $\mu$ l.

**23 mM cAMP solution:** Put 42.80 mg of cAMP (MW 329.22) in a 1.5 ml Eppendorf tube, add 73  $\mu$ l of Tris at 2 M (to bring the solution to pH 8.0). Finally, fill with water to 200  $\mu$ l.

**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  $\mu$ l of spermidine (MW 145.25) into a 1.5 Eppendorf tube and fill with water to 150  $\mu$ l.

**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  $\mu$ l of KOH (15 w/v %) .Finally, fill with water to 1.5 ml.

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 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 $\mu$ l
Nucleotide mix	396 $\mu$ l
NAD	76.6 $\mu$ l
CO-A	160 $\mu$ l
cAMP	46 $\mu$ l
Folinic acid	80 $\mu$ l
spermidine	34 $\mu$ l
Maltodextrin	2000 $\mu$ l
<b>TOTAL</b>	<b>3792.6 <math>\mu</math>l</b>

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. Keep 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**