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Energy solution preparation for OnePot PURE cell-free system [↗](#)Konstantinos Ragios¹¹EPFL - EPF Lausanne

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Works for me

[dx.doi.org/10.17504/protocols.io.8ayhsfw](https://doi.org/10.17504/protocols.io.8ayhsfw)

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ABSTRACT

In this protocol we explain the procedure to create the Energy solution used for protein expression in OnePot PURE cell-free system.

EXTERNAL LINK

<https://pubs.acs.org/doi/10.1021/acssynbio.8b00427>

MATERIALS TEXT

Material/Consumables:

- Amino Acids (Sigma-Aldrich: LAA21-1KT)
- Magnesium acetate (Sigma-Aldrich: M0631)
- Potassium glutamate (Sigma-Aldrich: 49601)
- DTT (SantaCruz Biotech: sc-29089B)
- ATP (ThermoFisher: R0481)
- GTP (ThermoFisher: R0481)
- CTP (ThermoFisher: R0481)
- UTP (ThermoFisher: R0481)
- tRNA (Roche:10109541001)
- Creatine phosphate (Sigma-Aldrich: 27920)
- Folinic acid (Sigma-Aldrich: PHR1541)
- Spermidine (Sigma-Aldrich: S2626)
- HEPES (Sigma-Aldrich: H0887-100ML)
- biDistilled water

Equipment:

- Vortex
- Nanodrop Spectrophotometer

BEFORE STARTING

Keep all the components in ice while preparing the Energy solution.

- 1 For a 2.5x Energy Solution add the materials needed to a tube. The final concentration of the components is presented in Table 1.



Before adding each component make sure it is totally melted and then vortex for a few seconds

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Compound	Concentration	Units
Amino Acids	0.75	mM
Magnesium acetate	29.5	mM
Potassium glutamate	250	mM
DTT	2.5	mM
ATP	5	mM
GTP	5	mM
CTP	2.5	mM
UTP	2.5	mM
tRNA	130	UA ₂₆₀ /mL
Creatine phosphate	50	mM
Folinic acid	0.05	mM
Spermidine	5	mM
HEPES	125	mM



Because tRNA degrades over time before adding it to the solution you need to check the A₂₆₀ units.

- 3 To complete the solution add biDistilled water until you reach the desirable volume, and then vortex for a few seconds
- 4 The Energy solution is stored at -80° C.



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