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Energy solution preparation for OnePot PURE cell-free system

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We use this protocol and it's working

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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.

Materials

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

Troubleshooting

Before start

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

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

Note

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	*UA260/mL*
	Creatine phosphate	50	*mM*

	Folinic acid	0.05	*mM*
	Spermidine	5	*mM*
	HEPES	125	*mM*

Note

Because tRNA degrades over time before adding it to the solution you need to check the A_{260} 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^{o} C.