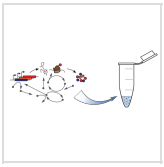

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Cell-free 3PGA energy solution

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1 Works for me dx.doi.org/10.17504/protocols.io.8pshvne

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ABSTRACT

Energy solution for E. coli lysate based on 3PGA. Adapted from Sun 2013 and Cai 2015. Successfully implemented at the University of Edinburgh by Nadanai Laohakunakorn, LBNC-EPFL by Zoe Swank.

Sun ZZ, Hayes CA, Shin J, Caschera F, Murray RM, Noireaux V (2013). Protocols for implementing an Escherichia coli based TX-TL cell-free expression system for synthetic biology.. Journal of visualized experiments : JoVE. https://doi.org/10.3791/50762

Cai Q, Hanson JA, Steiner AR, Tran C, Masikat MR, Chen R, Zawada JF, Sato AK, Hallam TJ, Yin G (2015). A simplified and robust protocol for immunoglobulin expression in Escherichia coli cell-free protein synthesis systems.. Biotechnology progress. https://doi.org/10.1002/btpr.2082

MATERIALS TEXT

- Amino acids LAA21-1KT Sigma
- Mg-glutamate 49605-250G Sigma
- K-glutamate 49601-500G Sigma
- DTT 10708984001 Sigma
- NTP set R1481 ThermoFisher
- tRNA 10109541001 Sigma
- CoA C4282-10MG Sigma
- NAD 10127981001 Sigma
- cAMP A9501-1G Sigma
- folinic acid PHR1541-1G Sigma
- spermidine S2626-1G Sigma
- PEG-8000 89510 Sigma
- 3PGA P8877-1G Sigma
- HEPES H3375-100G Sigma
- Tris base T1503-100G Sigma

- KOH
- mass balance

Amino acids stock solution

1 **Make stock solution of amino acids, 1000 uL at 50 mM.**

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1.1 Weigh each amino acid (excluding tyrosine) on parafilm paper, record the weight, and carefully add together in one tube. **Alternative: carrying out at 10x quantities makes weighing much easier.**

Amino acid	weight (mg)	added (mg)
Alanine	4.5	
Arginine	8.7	
Asparagine	6.6	
Aspartate	6.7	
Cysteine	6.1	
Glutamate	7.3	
Glutamine	7.3	
Glycine	3.8	
Histidine	7.8	
Isoleucine	6.6	
Leucine	6.6	
Lysine	9.1	
Methionine	7.5	
Phenylalanine	8.3	
Proline	5.8	
Serine	5.3	
Threonine	6.0	
Tryptophan	10.2	
Valine	5.9	

1.2 Add **1000 µl** of deionized water to the tube to make a 50 mM stock solution, vortex to mix, and adjust pH with KOH to ~5.2 (approximately 80 uL of 1M KOH and 920 uL dH20 required). pH can be measured roughly by spotting 1-2 uL of the solution on appropriate pH paper. If powder does not fully dissolve, add up to ~50 uL more of 1M KOH. pH will be ~8. Keep solution **On ice**

1.3 Weigh tyrosine and add to a separate tube

Amino acid	weight (mg)	added (mg)
Tyrosine	9.1	

1.4 Add **900 µl** of **1 Milimolar (mM)** KOH, and dissolve as far as possible. The tyrosine powder will not be entirely soluble.

1.5 Add **50 µl** of 15% KOH, which should fully dissolve the powder. Then add **50 µl** of deionized water. Vortex well, and measure pH, which should be around ~11-12. Keep solution **On ice**

1.6 Keep the tyrosine and the rest of the amino acids separate. If storage is required keep at **-80 °C** (flash-freezing with liquid nitrogen is optional)

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Other components stock solution

2 Make stock solution of other components

2.1 Prepare 2M stock solution of tris base.

Component	Mass to add (g)	Water to add (mL)	Final concentration
Tris base	60.57	250	2 M

2.2 Prepare 1M KOH stock, and 15% KOH stock.

2.3 Weigh out and make the following stock solutions. Four species require titration; their pH can be approximately measured by spotting 1 uL on appropriate pH paper.

Component	Mass to add (g)	Water to add (uL)	Tris to add (uL)	Final concentration	Notes
L-glutamic acid monopotassium salt (K-glutamate)	1.219	1000		6 M	
L-glutamic acid hemimagnesium salt (Mg-glutamate)	0.3886	1000		1 M	
DTT	0.1543	1000		1 M	
CoA	0.0498	1000		65 mM	
NAD	0.1161	to 1000	~90	175 mM	pH 7.5-8, titrate with 2M tris
Folinic acid	0.0160	1000		33.9 mM	
cAMP	0.2139	to 1000	~365	650 mM	pH 8, titrate with 2M tris
3-PGA	0.2604	to 1000	~540	1.4 M	pH 7.5, titrate with 2M tris
HEPES	0.4766	to 1000		2 M	pH 8, titrate with 1M KOH (around 25 uL required)
	Volume to add (uL)	**Water to add (uL)**		**Final concentration**	
spermidine	23.55	126.45		1 M	heat up stock solution in your hand
PEG-8000	50	50		50%	

Energy solution

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3 Preparation of final energy solution (4x)

This energy solution will form 25% of the final reaction volume.

3.1

Add the components together to produce the final energy solution, in the following order (not critical), vortexing after the addition of each one, and keeping tube **On ice**

Component	Stock (mM)	Final conc (mM)	Volume to add (uL)
HEPES	2000	200	100
Water			114.2
ATP	100	6	**60**
GTP	100	6	**60**
CTP	100	3.6	**36**
UTP	100	3.6	36
tRNA (in mg/ml)	43.75	0.8	18.29
CoA	65	1.04	16
NAD	175	1.32	7.54
cAMP	650	3	4.62
Folinic acid	33.9	0.27	8.02
Spermidine	1000	4	4
3-PGA	1400	120	85.7
amino acids	50	6	120
tyrosine	50	3	60
PEG-8000	50%	8%	160
Mg-glutamate	**1000**	**42**	**42**
K-glutamate	**6000**	**400**	**66.67**
DTT	1000	1	1
Total			**1000**

3.2

Measure and record pH of final solution using pH paper (should be ~8). Aliquot into storage tubes (25 uL recommended) and (optionally) flash freeze in liquid nitrogen.

3.3

Store at **-80 °C**

3.4

It is possible to calibrate the energy solution for maximum yield, in which case the optimization proceeds sequentially by determining optimum concentrations for Mg-glutamate, then K-glutamate. More details are given in Sun 2013.

- Sun 2013 report final optimal concentrations of 4.5-10.5 mM Mg-glutamate, 40-160 mM K-glutamate
- Kwon and Jewett 2015 report 12 mM Mg-glutamate, 130 mM K-glutamate

This step is not necessary if all that is required is functional extract.

For optimisation of energy solution, add all components apart from PEG, Mg-glu, K-glu. This makes a solution of **731.33 µl** and can be aliquoted into 10 tubes of **73.13 µl** .

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