Preparation of amino acid mixtures for cell-free expression systems

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Supplementary materials and methods

Cell-free extract preparation and cell-free reactions

The *E. coli* S30 extract preparation has presented before (1–3). Briefly, BL21 Rosetta2 cells are washed with S30A buffer: 14 mM Mg-glutamate, 60 mM K-glutamate, 50 mM Tris, and 2 mM DTT, buffered with glacial acetic acid to pH 8.2. The cytoplasm is extracted using a cell-press (12000 LB) and clarified by centrifugation at 30,000 × *g*. Then the cellular extract is dialyzed at 4°C for 1 h against the S30B buffer (14 mM Mg-glutamate and 150 mM K-glutamate, buffered to pH 8.2 with 2 M Tris) using cassettes with a 10 kDa cut-off. The crude extract represents 33% (30 µL) of the final reaction volume (90 µL), giving roughly 9.2 mg/mL of protein, and the remaining volume has the following components: water, 2 mM Mg-glutamate, 20 mM K-glutamate, 2% PEG-8000 (v/v), amino acids, maltodextrin at 216.186 g/L, and the reaction buffer (RB). The RB in the cell-free reactions has the following components: 50 mM Hepes pH 8, 1.5 mM ATP and GTP, 0.9 mM CTP and UTP, 0.2 mg/mL tRNA, 30 mM 3-PGA, 0.26 mM CoA, 0.33 mM NAD, 0.75 mM cAMP, 0.068 mM folinic acid, and 1 mM spermidine. All of the chemicals are purchased from Sigma-Aldrich. The deGFP synthesized is determined by fluorescent (excitation wavelength 485 nm, emission wavelength 528 nm) using a multimode plate reader H1m (BioTeK, Winooski, VT). The calibration line, used to retrieve the protein concentration is built with recombinant eGFP (reGFP) purchased from Cell Biolabs Inc. (San Diego, CA).

Solid amino acids

We used the kit LAA21–1KT from Sigma-Aldrich (St. Louis, MO). Four amino acids from the kit were not used (trans-4-hydroxy-l-proline, L-cysteine hydrochloride, L-histidine hydrochloride, L-lysine hydrochloride), and they were replaced by:

Name	Supplier	Code
L-proline	Sigma	81709–25G
L-cysteine	Sigma	30089–25G
L-histidine	Sigma	53319–25G
L-lysine	Sigma	L5501–5G

References

1. **Caschera, F. and V. Noireaux.** 2014. Synthesis of 2.3 mg/ml of protein with an all Escherichia coli cell-free transcription-translation system. Biochimie *99*:162-168.
2. **Sun, Z.Z., C.A. Hayes, J. Shin, F. Caschera, R.M. Murray, and V. Noireaux.** 2013. Protocols for Implementing an Escherichia coli Based TX-TL Cell-Free Expression System for Synthetic Biology. J Vis Exp. *79*:e50762.
3. **Shin, J. and V. Noireaux.** 2010. Efficient cell-free expression with the endogenous *E. coli* RNA polymerase and sigma factor 70. J Biol Eng. *4*:8.
4. **Lobry, J.R. and C. Gautier.** 1994. Hydrophobicity, expressivity and aromaticity are the major trends of amino-acid usage in 999 Escherichia coli chromosome-encoded genes. Nucleic Acids Res. *22*:3174-3180.

Supplementary Tables S1–S3 can be found on the next three pages.

Supplementary Table S1. Amino acid preparation at equimolar concentrations.

	MW	mM	µL AA mix	mM in KOH mixture
Alanine	89.09	4089	13.6	146
Arginine*	210.67	2314	22.2	135
Asparagine	132.12	3759	13.6	134
Aspartic acid	133.1	3752	13.6	134
Cysteine	121.16	2465	22.2	143
Glutamic acid	147.13	3655	13.6	130
Glutamine	146.15	2501	22.2	145
Glycine	75.07	4210	13.6	150
Histidine	155.15	3281	13.6	117
Isoleucine	131.18	3765	13.6	134
Leucine	131.18	2549	22.2	148
Lysine	146.19	2392	22.2	139
Methionine	149.21	2491	22.2	145
Phenylalanine	165.19	1716	34	153
Proline	115.13	3883	13.6	138
Serine	105.9	3953	13.6	141
Threonine	119.12	3853	13.6	137
Tryptophan	204.23	1661	34	148
Tyrosine	181.19	2396	22.2	139
Valine	117.15	2595	22.2	151

All of the solids (LAA21–1KT) are purchased from Sigma-Aldrich (St. Louis, MO) and dissolved according to the concentration listed in the table below in 500 µL 5 M KOH solution purchased from Sigma-Aldrich. Note that the final concentration of the amino acid stocks solution is calculated to account for a volume increase due to the physical mass of the solid. As an estimation, the volume increases is half the mass of the solid.
*Arginine monohydrocloride is dissolved in water. All of the other L-amino acids that have been used do not have counter ions.

To 381.6 µL of equimolar amino acids mixture at 2807 mM in 5 M KOH, water is added up to ~4 mL, and the pH is adjusted with glacial acetic acid:
- 100 µL acetic acid, pH = 8.49; the mixture is stable during aliquoting.
- 105 µL acetic acid, pH = 7.86; the mixture is stable during aliquoting.
- 110 µL acetic acid, pH = 6.51; the mixture is stable during aliquoting.

Supplementary Table S2. Amino acids preparation according to the *E. coli* distribution (4).

	E. coli distribution %	mM in KOH mixture	µL AA mix
Alanine	9.7	280	26
Arginine	5.3	154	25
Asparagine	3.6	104	11
Aspartic acid	4.8	139	14
Cysteine	1.1	31	5
Glutamic acid	5.4	156	16
Glutamine	6.2	177	27
Glycine	7.7	222	20
Histidine	1.9	54	6
Isoleucine	5.5	158	16
Leucine	10.8	311	47
Lysine	4.3	125	20
Methionine	2.9	84	13
Phenylalanine	4.1	119	27
Proline	4.2	122	12
Serine	5.5	158	15
Threonine	5.1	146	14
Tryptophan	1.5	44	10
Tyrosine	2.7	78	12
Valine	7.5	215	32

All of the solids (LAA21–1KT) are purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved according to the concentrations listed in the table below in 500 µL 5 M KOH solution purchased from Sigma-Aldrich. Amino acids are dissolved in 5 M KOH as described in Supplementary Table S1.
*Arginine monohydrocloride is dissolved in water. All the others L-amino acids that have been used do not have counter ions.

To 369 µL of *E. coli* amino acids mixture at 2878 mM in 5 M K(OH), water is added up to ~4 mL, and the pH adjusted with glacial acetic acid:
- 95 µL acetic acid, pH = 8.21; the mixture is stable during aliquoting.
- 100 µL acetic acid, pH = 7.51; the mixture is stable during aliquoting.
- 110 µL acetic acid, pH = 6.04; the mixture is stable during aliquoting.

Supplementary Table S3. Amino acids mixture preparation according to deGFP sequence.

	deGFP distribution %	mM in K(OH) mixture	µL AA mix
Alanine	3.7	105	10
Arginine	2.6	73	12
Asparagine	5.5	156	16
Aspartic acid	7.2	203	21
Cysteine	0.9	26	4
Glutamic acid	5.7	162	17
Glutamine	3.7	105	16
Glycine	9.5	267	24
Histidine	3.3	94	11
Isoleucine	5.1	142	14
Leucine	8.9	249	37
Lysine	7.9	223	36
Methionine	2.3	64	10
Phenylalanine	5.8	162	36
Proline	4.3	122	12
Serine	4	113	11
Threonine	6.5	184	18
Tryptophan	0.4	12	3
Tyrosine	4.4	123	20
Valine	8.2	230	34

The composition is retrieved from the gene sequence using http://www.expasy.org/. All of the solids (LAA21–1KT) are purchased from Sigma-Aldrich (St. Louis, MO) and dissolved according to the concentrations listed in the table below in 500 µL 5 M KOH solution purchased from Sigma-Aldrich. Amino acids are dissolved in 5 M KOH as described in Supplementary Table S1.
*Arginine monohydrocloride is dissolved in water. All of the other L-amino acids that have been used do not have counter ions.

To 360 µL of the *E. coli* amino acids mixture at 2812 mM in 5 M K(OH), water is added up to ~4 mL, and the pH is adjusted with glacial acetic acid:
- 95 µL acetic acid, pH = 8.72; the mixture is stable during aliquoting.
- 100 µL acetic acid, pH = 7.9; the mixture is stable during aliquoting.
- 105 µL acetic acid, pH = 6.82; the mixture is stable during aliquoting.