

Materials List for:

Protocols for Implementing an *Escherichia coli* Based TX-TL Cell-Free Expression System for Synthetic Biology

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Materials

| Name | Company | Catalog Number | Comments |
|--|-------------------|----------------|----------|
| 2xYT | MP biomedical | 3012-032 | |
| 3-PGA | Sigma-Aldrich | P8877 | |
| ATP | Sigma-Aldrich | A8937 | |
| Bacto-agar | BD Diagnostics | 214010 | |
| Bead-beating tubes (polypropylene microvials) | BioSpec | 522S | |
| Beads, 0.1mm dia. | BioSpec | 11079101 | |
| BL21 Rosetta 2 <i>E. coli</i> strain | Novagen | 71402 | |
| Bradford BSA Protein Assay Kit | Bio-rad | 500-0201 | |
| cAMP | Sigma-Aldrich | A9501 | |
| Chloramphenicol | Sigma-Aldrich | C1919 | |
| CoA | Sigma-Aldrich | C4282 | |
| CTP | USB | 14121 | |
| Cuvettes, 1.5ml | Fisher | 14-955-127 | |
| DTT | Sigma-Aldrich | D0632 | |
| Folinic acid | Sigma-Aldrich | F7878 | |
| GTP | USB | 16800 | |
| HEPES | Sigma-Aldrich | H6147 | |
| K-glutamate | Sigma-Aldrich | G1149 | |
| Mg-glutamate | Sigma-Aldrich | 49605 | |
| Micro Bio-Spin Chromatography Columns | Bio-Rad | 732-6204 | |
| NAD | Sigma-Aldrich | N6522 | |
| Nunc 384-well optical bottom plates | Thermo-Scientific | 142761 | |
| Nunc sealing tape | Thermo-Scientific | 232701 | |
| PEG-8000 | Promega | V3011 | |
| Potassium phosphate dibasic solution | Sigma-Aldrich | P8584 | |
| Potassium phosphate monobasic solution | Sigma-Aldrich | P8709 | |
| RTS Amino Acid Sampler | 5 Prime | 2401530 | |
| Slide-A-Lyzer Dialysis Cassettes, 10k MWCO (Kit) | Thermo-Scientific | 66382 | |

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|-----------------------------|-----------------------|------------|---|
| Spermidine | Sigma-Aldrich | 85558 | |
| Tris base | Fischer | BP1521 | |
| tRNA (from <i>E. coli</i>) | Roche Applied Science | MRE600 | |
| UTP | USB | 23160 | |
| 1L Centrifuge Bottle | Beckman-Coulter | A98813 | This is specific for Avanti J-series; obtain equivalent size for centrifuge in use. |
| 4L Erlenmeyer Flask | Kimble Chase | 26500-4000 | |
| Avanti J-26XP Centrifuge | Beckman-Coulter | 393127 | Or 1L-capable centrifuge equivalent. |
| Forma 480 Orbital Shaker | Thermo Scientific | 480 | Or chest-size 6x4L shaker equivalent. |
| JLA-8.1000 Rotor | Beckman-Coulter | 363688 | Or 1L-capable, 5000 x g rotor equivalent for centrifuge. |
| Mini-Beadbeater-1 | BioSpec | 3110BX | |

Supplemental Material 1. Recipes for Items.

Chloramphenicol, 34 mg/ml: Prepare 0.51 g chloramphenicol and add ethanol to 15 ml. Filter sterilize (0.22 µm), aliquot to 1 ml tubes, store at -20 °C for later use.

2xYT+P+Cm agar plate: Prepare 1.24 g 2xYT, 1.6 ml potassium phosphate dibasic solution @ 1 M, 0.88 ml potassium phosphate monobasic solution @ 1 M, 0.6 g agar, and water to 40 ml. Autoclave. Let cool to 50 °C and add 40 µl Cm. Aliquot 25 ml into a 100x15 mm petri dish, and let cool for an hour.

2xYT+P media: Prepare 124 g 2xYT, 160 ml potassium phosphate dibasic solution @1 M, 88 ml potassium phosphate monobasic solution @ 1 M, and water to 4 L. Aliquot out into 2x1.88 L and 0.24 L. Autoclave.

Tris base, 2 M: Prepare 60.57 g Tris base and water to 250 ml. Sterilize, store at RT for later use.

DTT, 1 M: Prepare 2.31 g DTT and water to 15 ml. Filter sterilize (0.22 µm), aliquot to 1 ml tubes, store at -20 °C for later use.

S30A buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, 50 ml Tris at 2M, acetic acid (to pH 7.7), and water to 2 L. Autoclave, store at 4 °C, add 4 ml 1 M DTT before use.

S30B buffer: Prepare 10.88 g Mg-glutamate and 24.39 g K-glutamate, Tris at 2 M (to pH 8.2), and water to 2 L. Autoclave, store at 4 °C, add 2 ml 1 M DTT before use.

HEPES: Prepare 1.91 g HEPES (MW 238.21), KOH (to pH 8), and water to 4 ml.

tRNA: Prepare 30 mg of tRNA and water to 600 µl.

CoA: Prepare 30 mg of CoA (MW 767.53) and water to 600 µl.

NAD: Add 34.83 mg of NAD (MW 663.43), Tris at 2 M (to pH 7.5-8), and water to 300 µl. (Add 27 µl of Tris at 2 M to bring the solution to pH 7.5-8).

cAMP: Add 42.80 mg of cAMP (MW 329.22), Tris at 2 M (to pH 8), and water to 200 µl. (Add 73 µl of Tris at 2 M to bring the solution to pH 8).

Folinic Acid (33.9 mM): To 20 mg of solid folinic acid calcium salt (MW 511.5), add 1.15 ml water.

Spermidine: Prepare 23.55 µl of spermidine (MW 145.25) and water to 150 µl. Prepare at room temperature after melting briefly at 37 °C.

3-PGA: Add 1.03 g of 3-PGA (MW 230.02), Tris at 2 M (to pH 7.5), and water to 3.2 ml. (Add 1.73 ml of Tris at 2 M to bring the solution to pH 7.5).

Nucleotide Mix: Add 145 mg of ATP dipotassium salt dihydrate (MW 619.4), 133 mg of GTP disodium salt (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), KOH at 15% dilution (to pH 7.5), and water to 1.5 ml. (Add 353 µl of KOH at 15% dilution to bring the solution to pH 7.5).

Supplemental Material 2. Bradford Assay.

1. Remove Bradford agent from 4 °C and set at room temperature.
2. Prepare 50 µl BSA Standard at 1 mg/ml and at 0.1 mg/ml.
3. Prepare 40 µl 20x dilution of extract from step 1.47.
4. Add 800 µl water to 7 cuvettes.
5. Prepare standard cuvettes for 0 mg/ml, 1 mg/ml (10 µl 0.1 mg/ml BSA), 2 mg/ml (20 µl 0.1 mg/ml BSA), 4 mg/ml (4 µl 1 mg/ml BSA), 6 mg/ml (6 µl 1 mg/ml BSA).
6. Prepare experimental cuvettes for 2 µl of sample and 4 µl of sample.
7. Add 200 µl of Bradford agent to each cuvette and mix well by pipetting. Incubate at room temperature for at least 10 min.
8. Produce standard curve at OD 595nm using cuvettes from step 6.5. Reject standard curve if $r^2 < 0.95$.
9. Determine extract concentration at OD 595nm using cuvettes from step 6.6.

Supplemental Material 3. Buffer calibration spreadsheet.

See [TXTL_e\(template\)_calibration_JoVE.xlsx](#).

Supplemental Material 4. Cell-free expression run spreadsheet.

See [TXTL_JoVE.xlsx](#).