

Materials List for:

Preparing Protein Producing Synthetic Cells using Cell Free Bacterial Extracts, Liposomes and Emulsion Transfer

Omer Adir^{*1,2}, Noga Sharf-Pauker^{*1,2}, Gal Chen^{*1,3}, Maya Kaduri¹, Nitzan Krinsky^{1,3}, Janna Shainsky-Roitman¹, Jeny Shklover¹, Avi Schroeder¹

¹Laboratory for Targeted Drug Delivery and Personalized Medicine Technologies, Department of Chemical Engineering, Technion - Israel Institute of Technology

²The Norman Seiden Multidisciplinary Program for Nanoscience and Nanotechnology, Technion - Israel Institute of Technology

³The Interdisciplinary Program for Biotechnology, Technion - Israel Institute of Technology

*These authors contributed equally

Correspondence to: Avi Schroeder at avids@technion.ac.il

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Materials

Name	Company	Catalog Number	Comments
A. Reagents required for step 1 (S30-T7 lysate preparation)			
E.coli BL21 (DE3)	NEB	C2527	E.coli BL21 (DE3).
pAR1219	Sigma	T2076	Targetron vector for transformation.
Stock solution of 50 mg/mL Ampicillin	Sigma	A9518	Stored at -20 °C.
10 g/L Bacto-tryptone	BD Bioscience	211705	For preparation of Luria Bertani (LB) agar (1.5%) plate.
10 g/L Sodium chloride (NaCl)	Bio-Lab	19030591	
5 g/L Bacto-Yeast extract	BD Bioscience	212750	
15 g/L Agar agar purified	Merck	1.01614.5007	
50 µg/mL Ampicillin	Sigma	A9518	
10 g/L Bacto-tryptone	BD Bioscience	211705	For preparation of Luria Bertani (LB) media (20 mL).
10 g/L Sodium chloride (NaCl)	Bio-Lab	19030591	
5 g/L Bacto-Yeast extract	BD Bioscience	212750	
50 µg/mL Ampicillin	Sigma	A9518	
12 g/L Bacto-tryptone	BD Bioscience	211705	For preparation of Terrific Broth (TB) media (1 L).
24 g/L Bacto-Yeast extract	BD Bioscience	212750	
4% (v/v) Glycerol anhydrous	Bio-Lab	7120501	
2.32 g/L K ₂ HPO ₄	Spectrum chemical	P1383	
12.54 g/L KH ₂ PO ₄	Spectrum chemical	P1380	
50 µg/mL Ampicillin	Sigma	A9518	
Stock solution of 100 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG)	INALCO	INA-1758-1400	Filtered using 0.2 µm hydrophilic PVDF syringe filter.
Stock solution of 0.1 M dithiothreitol (DTT)	TCI	D1071	Filtered using 0.2 µm hydrophilic PVDF syringe filter.
10 mM Tris-acetate at pH = 7.4	Sigma	T1503	S30 lysate buffer (1.5 L)
14 mM magnesium acetate	Merck	1.05819.0250	
60 mM potassium acetate	Carlo Erba	470147	
1 mM DTT	TCI	D1071	

0.5 mL/L 2-mercaptoethanol	Sigma	M6250	
Equipment required for step 1			
100 mL sterilized Erlenmeyer flasks	Thermo Scientific	50-154-2846	2 flasks
2 L sterilized Erlenmeyer flasks with baffles	KIMAX-KIMBLE	25630	2 flasks
Floor incubator shaker	MRC	TOU-120-2	Laboratory shaker incubator 450x450mm, 400rpm, 70 °C
Centrifuge	Thermo Scientific	75004270	(75003340) - Fiberlite F10-6 x 100 LEX Fixed-Angle Rotor. Should enable at least 13,000 x g. * Pre-cooled to 4 °C.
High pressure homogenizer	AVESTIN	EmulsiFlex-C3	Pre-cooled to 4 °C.
-80°C freezer	SO-LOW	U85-18	
Sterilized 1.5 mL plastic tubes	Eppendorf	30120086	Preferably pre-cooled to -20 °C.
Spectrophotometer	TECAN	IN-MNANO	Infinite M200 pro
96-well transparent plate	Thermo Scientific	167008	
Sterilized graduated cylinder	Corning		
Sterilized centrifuge tubes	Eppendorf	30120086	Preferably pre-cooled to -20 °C.
Sterilized pipette tips	Corning		Preferably pre-cooled to -20 °C.
Crushed ice bucket	Bel-Art	M18848-4001	
Small liquid nitrogen tank	NALGENE	4150-4000	
B. Reagents required for step 3 (lipids in oil solution preparation):			
1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)	Lipoid	556400	Powder
Cholesterol	Sigma	C8667	Powder
Chloroform	Bio-Lab	3082301	
Mineral oil	Sigma	M5904	Light oil
Equipment required for step 2			
Vortex mixer	Scientific industries	SI-0256	
Heating block	TECHNE	FDB03AD	Pre-heated to 80 °C. Should enable controlled temperature.
2 mL screw neck glass vials	CSI Analytical Innovations	VT009M-1232	For a larger scale, use 50 mL falcons and evaporate the chloroform using rotary evaporator.
9mm Screw Cap	CSI Analytical Innovations	C395R-09LC	
C. Reagents required for step 3 (inner and feeding reaction mixtures):			
HEPES	Spectrum	H1089	1 M HEPES-KOH (pH = 8) - pH buffer
Potassium hydroxide (KOH)	Frutarom	55290	
1 M Magnesium acetate	Merck	1.05819.0250	Co-factor and negative charge stabilizer.
1 M Potassium acetate	Carlo Erba	470147	Negative charge stabilizer.
5.2 M Ammonium acetate	Merck	1.01116.1000	Stabilizes negative charge.
50% (w/v) Polyethylene glycol 6000 (PEG)	Merck	8.07491.1000	Increases the concentration of the macromolecules.
0.5 M 3-phosphoglycerate (3-PGA)	Sigma	P8877	Secondary energy source.

50 mM Amino acids mixture I	Sigma	LAA21-1KT	Amino acids additive. Contains: 50 mM of each of the following 17 natural amino acids - alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, and valine.
50 mM Amino acids mixture II	Sigma	LAA21-1KT	Amino acids additive. Contains: 50 mM of each of the following 3 natural amino acids - tryptophan, phenylalanine, and tyrosine.
100 mM Adenine triphosphate (ATP)	Sigma	A3377	Nucleotides & energy source.
50 mM Guanidine triphosphate (GTP)	Sigma	G8877	Nucleotides & energy source.
100 mM Uridine triphosphate (UTP)	ACROS ORGANICS	226310010	Nucleotides additive.
100 mM Isopropyl β -D-1-thiogalactopyranoside (IPTG)	INALCO	INA-1758-1400	Genes expression induction.
2 M Sucrose	J.T. Baker	1933078	Generating a density gradient.
2 M Glucose	Sigma	16301	Generating a density gradient.
H ₂ O UltraPure Water (UPW)	Bio-Lab	2321777500	DNase & RNase free
S30-T7 lysate	–	–	Prepared at step 1. Source of transcription & translation components. Store at -80 °C, thaw on crushed ice just before usage.
Stock of DNA plasmid of choice	–	–	Contains the sequence for the requested protein. Under T7 promotor
D. Equipment required for step 4 (synthetic cells preparation)			
Floor incubator shaker or Thermomixer	MRC	TOU-120-2	Laboratory shaker incubator 450x450mm, 400rpm, 70 °C
PHMT Grant Bio	PSC18	Thermomixer	
Centrifuge	Thermo Scientific	75004270	(75003629) - TX-400 4 x 400mL Swinging Bucket Rotor. Suited for 15 mL sized tubes. Preferably swinging buckets. Should enable at least 1000 x g. Pre-cooled to 4 °C.
Table centrifuge	Thermo Scientific	75002420	(75003424) - 24 x 1.5/2.0mL rotor with ClickSeal. Suited for Eppendorf vials. Pre-cooled to 4 °C.
Vortex mixer	Scientific industries	SI-0256	
Crushed ice bucket	Bel-Art	M18848-4001	
2 mL screw neck glass vials	CSI Analytical Innovations	VT009M-1232	
Sterile 15 mL plastic tubes	Thermo Scientific	339651	
Sterilized 1.5 mL plastic tubes	Eppendorf	30120086	
Sterilized pipette tips	Corning		Sterilized by autoclave.