

Materials List for:

Genetic Incorporation of Biosynthesized L-dihydroxyphenylalanine (DOPA) and Its Application to Protein Conjugation

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Materials

Name	Company	Catalog Number	Comments
1. Plasmid Construction			
Plasmid pBAD-dual-TPL-GFP-E90TAG			optionally contain the amber stop codon(TAG) at a desired position. Ko, W. et al. Efficient and Site-Specific Antibody Labeling by Strain-promoted Azide-Alkyne Cycloaddition. BKCS. 36 (9), 2352-2354, doi: 10.1002/bkcs.10423, (2015)
Plasmid pEvol-DHPRS2			1. Young, T. S., Ahmad, I., Yin, J. A., and Schultz, P. G. An enhanced system for unnatural amino acid mutagenesis in E. coli. J. Mol. Biol. 395 (2), 361-374, doi: 10.1016/j.jmb.2009.10.030, (2010) 2. Kim, S., Sung, B. H., Kim, S. C., Lee, H. S. Genetic incorporation of L-dihydroxyphenylalanine (DOPA) biosynthesized by a tyrosine phenol-lyase. Chem. Commun. 54 (24), 3002-3005, doi: 10.1039/c8cc00281a (2018).
DH10β	Invitrogen	C6400-03	Expression Host
Plasmid Mini-prep kit	Nucleogen	5112	200/pack
Agarose	Intron biotechnology	32034	500 g
Ethidium bromide	Alfa Aesar	L07482	1 g
LB Broth	BD Difco	244620	500 g
2. Culture preparation			
2.1) Electroporation			
Micro pulser	BIO-RAD	165-2100	
Micro pulser cuvette	BIO-RAD	165-2089	0.1 cm electrode gap, pkg. of 50
Ampicillin Sodium	Wako	018-10372	25 g
Chloramphenicol	Alfa Aesar	B20841	25 g
Agar	SAMCHUN	214230	500 g
SOC medium	Sigma	S1797	100 mL
3. Expression and Purification of GFP-E90DOPA by biosynthetic system			
3.1 Expression of GFP-E90DOPA by biosynthetic system			
L(+)-Arabinose, 99%	Acros	104981000	100 g

Pyrocatechol, 99%	SAMCHUN	P1387	25 g
Ammonium sulfate, 99%	SAMCHUN	A0943	500 g
pyruvic acid, 98%	Alfa Aesar	A13875	100 g
Sodium phosphate dibasic, anhydrous, 99%	SAMCHUN	S0891	1 kg
Potassium phosphate, monobasic, 99%	SAMCHUN	P1127	1 kg
Magnesium sulfate, anhydrous, 99%	SAMCHUN	M0146	1 kg
D(+)-Glucose, anhydrous, 99%	SAMCHUN	D0092	500 g
Glycerol, 99%	SAMCHUN	G0269	1 kg
Trace metal mix a5 with co	Sigma	92949	25 mL
L-Proline, 99%	SAMCHUN	P1257	25 g
L-Phenylalanine, 98.5%	SAMCHUN	P1982	25 g
L-Tryptophane	JUNSEI	49550-0310	25 g
L-Arginine, 98%	SAMCHUN	A1149	25 g
L-Glutamine, 98%	JUNSEI	27340-0310	25 g
L-Asparagine monohydrate, 99%	SAMCHUN	A1198	25 g
L-Methionine	JUNSEI	73190-0410	25 g
L-Histidine hydrochloride monohydrate, 99%	SAMCHUN	H0604	25 g
L-Threonine, 99%	SAMCHUN	T2938	25 g
L-Leucine	JUNSEI	87070-0310	25 g
Glycine, 99%	SAMCHUN	G0286	25 g
L-Glutamic acid, 99%	SAMCHUN	G0233	25 g
L-Alanine, 99%	SAMCHUN	A1543	25 g
L-Isoleucine, 99%	SAMCHUN	I1049	25 g
L-Valine, 99%	SAMCHUN	V0088	25 g
L-Serine	SAMCHUN	S2447	25 g
L-Aspartic acid	SAMCHUN	A1205	25 g
L-Lysine monohydrochloride, 99%	SAMCHUN	L0592	25 g
3.2 Cell lysis			
Imidazole, 99%	SAMCHUN	I0578	1kg
Sodium phosphate monobasic, 98%	SAMCHUN	S0919	1 kg
Sodium Chloride, 99%	SAMCHUN	S2907	1 kg
Ultrasonic Processor - 150 microliters to 150 milliliters	SONIC & MATERIALS	VCX130	
3.3 Ni-NTA Affinity Chromatography			
Ni-NTA resin	QIAGEN	30210	25 mL
Polypropylene column	QIAGEN	34924	50/pack, 1 mL capacity
4. Oligomerization of Purified GFP-E90DOPA			
Sodium periodate, 99.8%	Acros	419610050	5 g
5. Conjugation of GFP-E90DOPA with an Alkyne Probe by Strain-Promoted Oxidation-Controlled Cyclooctyne-1,2-Quinone Cycloaddition (SPOCQ)			

Cy5.5-ADIBO	FutureChem	FC-6119	1mg
6. Purification of Labeled GFP			
Amicon Ultra 0.5 mL Centrifugal Filters	MILLIPORE	UFC500396	96/pack, 500ul capacity
7. SDS-PAGE Analysis and Fluorescence Gel Scanning			
1,4-Dithio-DL-threitol, DTT, 99.5 %	Sigma	10708984001	10 g
NuPAGE LDS Sample Buffer, 4X	ThermoFisher	NP0007	10 mL
MES running buffer	ThermoFisher	NP0002	500 mL
Nupage Novex 4-12% SDS PAGE gels	ThermoFisher	NO0321	12 well
Coomassie Brilliant Blue R-250	Wako	031-17922	25 g
G:BOX Chemi Fluorescent & Chemiluminescent Imaging System	Syngene		G BOX Chemi XT4
8. MALDI-TOF MS analysis by Trypsin Digestion			
8.1 Preparation of the digested peptide sample by trypsin digestion			
Tris(hydroxymethyl)aminomethane, 99%	SAMCHUN	T1351	500 g
Hydrochloric acid, 35~37%	SAMCHUN	H0256	500 mL
Dodecyl sulfate sodium salt, 85%	SAMCHUN	D1070	250 g
Iodoacetamide	Sigma	I6125	5 g
Trypsin Protease, MS Grade	ThermoFisher	90057	5 x 20 µg/pack
C-18 spin columns	ThermoFisher	89870	25/pack, 200 µL capacity
8.2 Analysis of the digested peptide by MALDI-TOF			
Acetonitrile, 99.5%	SAMCHUN	A0125	500 mL
α-Cyano-4-hydroxycinnamic acid	Sigma	C2020	10 g
Trifluoroacetic acid, 99%	SAMCHUN	T1666	100 g
MTP 384 target plate ground steel BC targets	Bruker	8280784	
Bruker Autoflex Speed MALDI-TOF mass spectrometer	Bruker		