

Materials List for:

# Residue-specific Incorporation of Noncanonical Amino Acids into Model Proteins Using an *Escherichia coli* Cell-free Transcription-translation System

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## Materials

Name	Company	Catalog Number	Comments
Protective eyewear	Sigma-Aldrich, St. Louis, USA	Z758841	
Nitrile gloves (size S)	Sigma-Aldrich, St. Louis, USA	Z768960	Catalog numbers other sizes: Z768979 for M, Z768987 for L and Z768995 for XL
Eppendorf Safe-Lock Tube 1.5 ml (PCR clean)	Eppendorf, Hamburg, Germany	30123.328	
Microbalance Discovery DV114CM	Ohaus, Greifensee, Switzerland	80104140	
Microspatula (L 6 5/8 in., stainless steel, rod diam. 0.09 in.)	Sigma-Aldrich, St. Louis, USA	Z243213	
L-Canavanine	Sigma-Aldrich, St. Louis, USA	C9758	Acute toxicity: wear eyeshields, dust mask, protective gloves
Hydroxylysine (racemic mixture)	Sigma-Aldrich, St. Louis, USA	H0377	
Cryo-gloves (size S, water resistant)	Sigma-Aldrich, St. Louis, USA	Z183490	Catalog numbers other sizes: Z183512 for M, Z183520 for L and Z183539 for XL
RTS Amino Acid Sampler	Biotechrabbit, Hennigsdorf, Germany	BR1401801	For homemade preparation of amino acid stock solutions, follow this protocol <sup>35</sup> and use the solid amino acid kit LAA21-1KT, L-proline (81709-25G), L-cysteine (30089-25G), L-histidine (53319-25G) and L-lysine (L5501-5G) (all from Sigma-Aldrich, St. Louis, USA)
HEPES	Sigma-Aldrich, St. Louis, USA	H6147	
ATP	Sigma-Aldrich, St. Louis, USA	A8937	
CTP	Affymetrix, Santa Clara, USA	14121	
GTP	Affymetrix, Santa Clara, USA	16800	
UTP	Affymetrix, Santa Clara, USA	23160	
tRNA (from <i>E. coli</i> , pack size 100 mg)	Sigma-Aldrich, St. Louis, USA	10109541001	Catalog number for pack size of 500 mg is 10109550001
CoA	Sigma-Aldrich, St. Louis, USA	C4282	
NAD (from yeast)	Sigma-Aldrich, St. Louis, USA	N6522	
cAMP	Sigma-Aldrich, St. Louis, USA	A9501	
Folinic acid	Sigma-Aldrich, St. Louis, USA	F7878	
3-PGA	Sigma-Aldrich, St. Louis, USA	P8877	
Mg-glutamate	Sigma-Aldrich, St. Louis, USA	49605	
K-glutamate	Sigma-Aldrich, St. Louis, USA	G1149	

pBEST-OR2-OR1-Pr-UTR1-deGFP-T500	Addgene, Cambridge, USA	Plasmid #40019	
4-20% precast Tris-Glycine Gels (10 cm x 10 cm x 1 mm, 10 courses)	Anamed Elektrophorese, Groß-Bieberau / Rodau, Germany	TG 81610	
SDS running buffer (10 x concentrate, 5,000 ml)	Anamed Elektrophorese, Groß-Bieberau / Rodau, Germany	TG 50001	
SDS loading buffer (2 x concentrate, 50 ml)	Anamed Elektrophorese, Groß-Bieberau / Rodau, Germany	TG 05002	
Unstained protein marker, broad range (2-212 kDa)	New England Biolabs, Ipswich, USA	P7702S	
Methanol	Merck, Darmstadt, Germany	1060091011	Toxic by inhalation, in contact with skin and if swallowed: wear protective gloves and work under fume hood
Acetic acid (99.8%)	VWR International, Darmstadt, Germany	20104.447	
Coomassie Blue G-250 (10 g)	Biozym Scientific, Hessisch Oldendorf, Germany	902120	
His-Spin Protein Miniprep kit	Zymo Research Europe, Freiburg, Germany	P2002	Product also distributed by Zymo Research Corporation, Irvine, USA
Trizma Base	Sigma-Aldrich, St. Louis, USA	T1503	
Hydrochloric acid	Sigma-Aldrich, St. Louis, USA	H1758	
Glycerol, 99%	VWR International, Darmstadt, Germany	24397.296DB	
CentriPure Z25 mini spin columns	Genaxxon bioscience, Ulm, Germany	CP-0205-Z100	
Sodium chloride	Sigma-Aldrich, St. Louis, USA	S9888	
Concentrator 5301	Eppendorf, Hamburg, Germany	5301 000.210	
2xYT	MP biomedicals, Santa Ana, USA	113012032	
Bacto-Agar	BD Diagnostics, Franklin Lakes, USA	214010	
Bead-beating tubes (polypropylene microvials)	Biospec, Bartlesville, USA	522S	
Beads, 0.1 mm dia.	Biospec, Bartlesville, USA	11079101	
BL21 Rosetta 2 <i>E. coli</i> strain	Merck, Darmstadt, Germany	71402	
Bradford BSA protein assay Kit	Bio-Rad, München, Germany	500-0201	
Chloramphenicol	Sigma-Aldrich, St. Louis, USA	C1919	
Cuvettes, 1.5 ml	Thermo Fisher Scientific, Waltham, USA	14-955-127	
DTT	Sigma-Aldrich, St. Louis, USA	D0632	
Micro Bio-Spin Chromatography Columns	Bio-Rad, München, Germany	732-6204	
Nunc 384-well optical bottom plates	Thermo Fisher Scientific, Waltham, USA	142761	
Nunc sealing tape	Thermo Fisher Scientific, Waltham, USA	232701	
PEG-8000	Promega, Madison, USA	V3011	
Potassium phosphate dibasic solution	Sigma-Aldrich, St. Louis, USA	P8584	
Potassium phosphate monobasic solution	Sigma-Aldrich, St. Louis, USA	P8709	

Slide-A-Lyzer dialysis cassettes, 10k MWCO (Kit)	Thermo Fisher Scientific, Waltham, USA	66382	
Spermidine	Sigma-Aldrich, St. Louis, USA	85558	
1 L centrifuge bottle	Beckman-Coulter, Brea, USA	A98813	
4 L Erlenmeyer flask	Kimble Chase, Vineland (NJ), USA	26500-4000	
Avanti J-26XP centrifuge	Beckman-Coulter, Brea, USA	393127	Or equivalent centrifuge able to centrifuge 1 L bottles.
Forma 480 orbital shaker	Thermo Fisher Scientific, Waltham, USA	480	Or equivalent shaker able to shake chest-size 6 x 4 L .
JLA-8.1000 rotor	Beckman-Coulter, Brea, USA	363688	Or equivalent 5,000 x g rotor for the centrifuge above, able to centrifuge 1 L bottles.
Mini-Beadbeater-1	Biospec, Bartlesville, USA	3110BX	
Microfuge 22R refrigerated microcentrifuge	Beckman-Coulter, Brea, USA	368831	Or equivalent centrifuge able to centrifuge 2 ml reaction tubes.
Heating block HLC HBT 130	Labexchange, Burladingen, Germany	24465	Or equivalent heating block able to heat samples in reaction tubes up to 100 °C.
Eppendorf MiniSpin centrifuge	Eppendorf, Hamburg, Germany	5452000018	Or equivalent centrifuge able to centrifuge 2 ml reaction tubes.
IKA Vortex 3 (4 mm orbital shaker diameter, 0 - 2,500 rpm)	Sigma-Aldrich, St. Louis, USA	Z654760	Or equivalent vortex.
Scotsman AF103 ice flaker machine	Kälte-Berlin, Berlin, Germany	AF103	Or equivalent ice flaker machine.
MyTemp mini digital incubator	Sigma-Aldrich, St. Louis, USA	Z763314	Or equivalent incubator able to heat samples at 29 °C.
EcoCell electrophoresis cell / chamber	Anamed Elektrophorese, Groß-Bieberau / Rodau, Germany	AN12005	Or equivalent electrophoresis chamber able to perform vertical gel electrophoresis with the above precast gels or other gels used.
Power-phor power supply for electrophoresis cell / chamber	Anamed Elektrophorese, Groß-Bieberau / Rodau, Germany	AN12001	Or equivalent power supply able to supply above used electrophoresis cell / chamber with power
VWR Signature Ergonomic High Performance Single-Channel Variable Volume Pipettors Starter Kit 1	VWR International, Darmstadt, Germany	613-5278	Or equivalent micropipettes enabling to pipette similar volumes with the same precision
VWR Signature Ergonomic High Performance Single-Channel Variable Volume Pipettors Starter Kit 2	VWR International, Darmstadt, Germany	613-5279	Or equivalent micropipettes enabling to pipette similar volumes with the same precision
Pipetman Tips Diamond D10ST (0.1 - 10 µl)	Gilson, Middleton, USA	F171101	Or equivalent low-binding pipette tips enabling to pipette similar volumes with the same precision
Pipetman Tips Diamond D200ST (2 - 200 µl)	Gilson, Middleton, USA	F171301	Or equivalent low-binding pipette tips enabling to pipette similar volumes with the same precision
Pipetman Tips Diamond D1000ST (100 - 1,000 µl)	Gilson, Middleton, USA	F171501	Or equivalent low-binding pipette tips enabling to pipette similar volumes with the same precision
50 ml centrifuge tubes with screw caps (sterile)	VWR International, Darmstadt, Germany	50-0156	
15 ml centrifuge tubes with screw caps (sterile)	VWR International, Darmstadt, Germany	525-0150	
14 ml polypropylene tubes (round bottom, two-position vent stopper, sterile)	Greiner Bio-One, Frickenhausen, Germany	187262	

Discovery BIO Wide Pore C5 HPLC Column (3 µm particle size, L x I.D. 10 cm x 2.1 mm)	Sigma-Aldrich, St. Louis, USA	567227-U	
Agilent 1260 HPLC machine	Agilent Technologies, Santa Clara, USA	G1312B	
6500 Series Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS	Agilent Technologies, Santa Clara, USA	G6530BA	
Acetonitrile	Sigma-Aldrich, St. Louis, USA	270717	
FLUOstar Omega microplate reader	BMG Labtech, Ortenberg, Germany	415-101	Or equivalent microplate reader able to measure the fluorescence of the expressed model protein
Hanna Checker pH meter	Sigma-Aldrich, St. Louis, USA	Z351091	
Formic acid eluent additive for LC-MS	Sigma-Aldrich, St. Louis, USA	56302	