

Residue-Specific Exchange of Proline by Proline Analogs in Fluorescent Proteins: How "Molecular Surgery" of the Backbone Affects Folding and Stability

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Materials

Name	Company	Catalog Number	Comments
Acetonitrile	VWR	HiPerSolv CHROMANORM ULTRA for LC-MS, 83642	LC-MS grade required
Acrylamide and bisacrylamide aqueous stock solution at a ratio of 37.5:1 (ROTIPHORESE Gel 30)	Carl Roth	3029.1	
Agar-agar	Carl Roth	5210	
Ammonium molybdate ((NH ₄) ₂ MoO ₄)	Sigma-Aldrich	277908	
Ammonium peroxydisulphate (APS)	Carl Roth	9592.2	≥98 %, p.a., ACS grade required
Ammonium sulfate ((NH ₄) ₂ SO ₄)	Sigma-Aldrich	A4418	
Ampicillin sodium salt	Carl Roth	K029	
Biotin	Sigma-Aldrich	B4501	
Bromophenol blue	Sigma-Aldrich	B0126	
Calcium chloride (CaCl ₂)	Sigma-Aldrich	C5670	
Coomassie Brilliant Blue R 250	Carl Roth	3862	
Copper sulfate (CuSO ₄)	Carl Roth	CP86.1	
D-glucose	Carl Roth	6780	
1,2-Bis-(dimethylamino)-ethane, N,N,N',N'-Tetramethylethylenediamine (TEMED)	Carl Roth	2367.3	≥99 %, p.a., for electrophoresis
1,4-dithiothreitol (DTT)	Carl Roth	6908	
Dichloromethane (DCM)	Sigma-Aldrich	270997	
di-potassium hydrogen phosphate (K ₂ HPO ₄)	Carl Roth	P749.1	

di-sodium hydrogen phosphate (Na ₂ HPO ₄)	Carl Roth	X987	
DNase I	Sigma-Aldrich	D5025	
Dowex 50WX8-100 (hydrogen form)	Acros Organics / Thermo Fisher Scientific (Waltham, U.S.A.)	10731181	cation exchange resin
Ethanol	Carl Roth	9065.1	
Formic acid	VWR	HiPerSolv CHROMANORM for LC-MS, 84865	LC-MS grade required
Glacial acetic acid	Carl Roth	3738.5	100 %, p. a.
Glycerol	Carl Roth	3783	
Imidazole	Carl Roth	X998	
Hydrogen chloride (HCl)	Merck	295426	
Iron(II) chloride (FeCl ₂)	Sigma-Aldrich	380024	
Isopropanol	Carl Roth	AE73.1	
Isopropyl β-D-1-thiogalactopyranoside (IPTG)	Sigma-Aldrich	I6758	
Lysozyme	Sigma-Aldrich	L6876	
Magnesium chloride (MgCl ₂)	Carl Roth	KK36.1	
Magnesium sulfate (MgSO ₄)	Carl Roth	8283.2	
Manganese chloride (MnCl ₂)	Sigma-Aldrich	63535	
β-mercaptoethanol	Carl Roth	4227.3	
PageRuler Unstained Protein Ladder	Thermo Fisher Scientific	26614	
Potassium chloride (KCl)	Carl Roth	6781.3	
Potassium dihydrogen phosphate (KH ₂ PO ₄)	Sigma-Aldrich	P5655	
RNase A	Carl Roth	7156	
Sodium chloride (NaCl)	Carl Roth	P029	
Sodium dihydrogen phosphate (NaH ₂ PO ₄)	Carl Roth	T879	
Sodium dodecyl sulphate (NaC ₁₂ H ₂₅ SO ₄)	Carl Roth	0183	
Thiamine	Sigma-Aldrich	T4625	
Trifluoroacetic acid (TFA)	Sigma-Aldrich	T6508	
Tris hydrochloride (Tris-HCl)	Sigma-Aldrich	857645	
Tris(hydroxymethyl)-aminomethane (Tris)	Carl Roth	5429	
Tryptone	Carl Roth	8952	
Yeast extract	Carl Roth	2363	
Zinc chloride (ZnCl ₂)	Sigma-Aldrich	229997	
L-alanine	Sigma-Aldrich	A7627	
L-arginine	Sigma-Aldrich	A5006	
L-asparagine	Sigma-Aldrich	A8381	
L-aspartic acid	Sigma-Aldrich	A0884	
L-cysteine	Sigma-Aldrich	C7352	
L-glutamic acid	Sigma-Aldrich	G2128	
L-glutamine	Sigma-Aldrich	G3126	
L-glycine	Sigma-Aldrich	G7126	
L-histidine	Sigma-Aldrich	H8000	
L-isoleucine	Sigma-Aldrich	I2752	

L-leucine	Sigma-Aldrich	L8000	
L-lysine	Sigma-Aldrich	L5501	
L-methionine	Sigma-Aldrich	M9625	
L-phenylalanine	Sigma-Aldrich	P2126	
L-proline	Sigma-Aldrich	P0380	
L-serine	Sigma-Aldrich	S4500	
L-threonine	Sigma-Aldrich	T8625	
L-tryptophan	Sigma-Aldrich	T0254	
L-tyrosine	Sigma-Aldrich	T3754	
L-valine	Sigma-Aldrich	V0500	
(4S)-fluoroproline	Bachem	4033274	Make sure that all proline analogs are proline free, check content. Otherwise include a step to consume proline contaminations during expression.
(4R)-fluoroproline	Bachem	4033275	Make sure that all proline analogs are proline free, check content. Otherwise include a step to consume proline contaminations during expression
3,4-dehydroproline	Bachem	4003545	Make sure that all proline analogs are proline free, check content. Otherwise include a step to consume proline contaminations during expression
4,4-difluoroproline	Enamine	EN400-17448	Make sure that all proline analogs are proline free, check content. Otherwise include a step to consume proline contaminations during expression
Conical polystyrene (Falcon) tubes, 15 mL	Fisher Scientific	14-959-49B	
Conical polystyrene (Falcon) tubes, 50 mL	Fisher Scientific	14-432-22	
Dialysis membrane, Molecular Weight Cut-Off (MWCO) 5,000	Spectrum Medical Industries	Spectra/Por MWCO 5000 dialysis membrane, 133198	
Immobilized Metal ion Affinity Chromatography (IMAC) column 1 mL, Ni-NTA	GE Healthcare	HisTrap HP, 1 mL, 17-5247-01	
Luer-Lock syringe, 5 mL	Carl Roth	EP96.1	
Luer-Lock syringe, 20 mL	Carl Roth	T550.1	
Luer-Lock syringe, 50 mL	Carl Roth	T552.1	
Microcentrifuge tubes, 1.5 mL	Eppendorf	30120086	
Petri dishes (polystyrene, sterile)	Carl Roth	TA19	
pQE-80L plasmid vector	Qiagen	no longer available	replaced by N-terminus pQE Vector set Cat No./ID: 32915
Pro-auxotrophic <i>E. coli</i> strain JM83	Addgene	50348	https://www.addgene.org/50348/
Pro-auxotrophic <i>E. coli</i> strain JM83	ATCC	35607	
Round-bottom polystyrene tubes, 14 mL	Fisher Scientific	Corning Falcon, 14-959-1B	
Syringe filter 0.45 µm with polyvinylidene difluoride (PVDF) membrane	Carl Roth	CCY1.1	
High-Performance Liquid Chromatography (HPLC) column for LC-ESI-TOF-MS	Sigma-Aldrich	Supelco Discovery BIO Wide Pore C5 HPLC column, 3 µm particle size, 10 cm x 2.1 mm	with conical 0.1 mL glass inserts, screw caps and septa
HPLC autosampler vials 1.5 mL	Sigma-Aldrich	Supelco 854165	

Mass spectrometer for LC-ESI-TOF-MS	Agilent	Agilent 6530 Accurate-Mass QTOF	
Mass spectrometry data analysis software	Agilent	MassHunter Qualitative Analysis software v. B.06.00	
Benchtop centrifuge for 1.5 mL Eppendorf tubes	Eppendorf	5427 R	
Cooling centrifuge for 50 mL Falcon tubes	Eppendorf	5810 R	
Fast Protein Liquid Chromatography (FPLC) system	GE Healthcare	ÄKTA pure 25 L	
Fluorescence spectrometer	Perkin Elmer	LS 55	
High pressure microfluidizer for bacterial cell disruption	Microfluidics	LM series with “Z” type chamber	
Orbital shaker for bacterial cultivation	Infors HT	Minitron	
Peristaltic pump for liquid chromatography (LC)	GE Healthcare	P-1	
Ultrasonic homogenizer for bacterial cell disruption	Omnilab	Bandelin SONOPULS HD 3200, 5650182	with MS72 sonifier tip
UV-Vis spectrophotometer	Biochrom	ULTROSPEC 2100	
UV-Vis/NIR spectrophotometer	Perkin Elmer	LAMBDA 950 UV/Vis/NIR	