

Materials List for:

Thermostabilization, Expression, Purification, and Crystallization of the Human Serotonin Transporter Bound to S-citalopram

Jonathan A. Coleman¹, Evan M. Green², Eric Gouaux^{1,3}

¹Vollum Institute, Oregon Health & Science University

²Graduate Group in Biophysics, University of California, San Francisco

³Howard Hughes Medical Institute, Oregon Health & Science University

Correspondence to: Eric Gouaux at gouauxe@ohsu.edu

URL: <https://www.jove.com/video/54792>

DOI: [doi:10.3791/54792](https://doi.org/10.3791/54792)

Materials

Name	Company	Catalog Number	Comments
DH10Bac	Invitrogen	10361-012	
Kanamycin	Fisher	BP906-5	
Gentamicin	Fisher	BP918-1	
Tetracycline	Sigma	T-7660	
Bluo-gal	Invitrogen	15519-028	5-Bromo-3-indolyl β -D-galactopyranoside
Isopropyl beta-D-1-thiogalactopyranoside	Anatrace	I1003	IPTG
Miniprep kit	Qiagen	27106	
Cellfectin II	Invitrogen	10362-100	Sf9 transfection reagent
Sf9	ATCC	CRL-1711	
Sf-900 III SFM media	Life Technologies	12658-027	
HEK-293S GnTI ⁻	ATCC	CRL-3022	
Freestyle 293 media	Life Technologies	12338-018	293 expression media
Fetal Bovine Serum (FBS)	Life Technologies	0984018DJ	
Sodium butyrate	Sigma	303410	
S-citalopram	Sigma	E4786	Anagrade
n-Dodecyl-beta-D-Maltopyranoside	Anatrace	D310	
Cholesteryl hemmisuccinate	Sigma	C6013	
1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphocholine	Avanti Polar Lipids	850457P	
1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphoethanolamine	Avanti Polar Lipids	850757P	
1-palmitoyl-2-oleoyl- <i>sn</i> -glycero-3-phosphoglycerol	Avanti Polar Lipids	840457P	
Leupeptin	Sigma	L2884	
Pepstatin A	Sigma	P5318	
Aprotinin	Sigma	A1153	
PMSF	Sigma	P7626	
Desthiobiotin	Iba Life Sciences	2-1000-05	
Asolectin	Sigma	11145	
Cholesterol	Sigma	C-8667	
Lipid A	Sigma	L5399	
Brain polar lipid	Avanti Polar Lipids	141101C	

Biobeads	Biorad	152-3920	Hydrophobic absorption resin
Goat anti-mouse IRDye 680RD	Odyssey	926-68070	Used as secondary antibody for western blotting
Lauryl maltose neopentyl glycol	Anatrace	NG310	Anagrade
Serotonin	Sigma	H9523	
pFastBac 8B6			Available from authors
pEG Bacmam SERT Strep II			SERT _{IC} , Available from authors
pEG Bacmam SERT GFP twin Strep His			SERT _{TC} , Available from authors
pEG Bacmam SERT ts3 GFP twin Strep His			SERT _{CC} , Available from authors
Imidazole	Sigma	56749	
n-Octyl β -D-maltoside	Anatrace	O310	Anagrade
Thrombin	Haematologic Technologies	HCT-0020	
EndoH	New England Biolabs	P0702	
Trizma-HCl	Sigma	T5941	Tris is used for preparation of crystallization reservoirs
PEG 400	Sigma	91893	
6-aminohexanoic acid	Sigma	7260	
Trypsin-EDTA	Fisher	MT25052CV	
Isoplate-96 TC	PerkinElmer	6005070	
PolyJet	SignaGen	SL100688	Polymer transfection reagent for mamalian cells
Copper HIS-Tag YSI SPA Beads	PerkinElmer	RPNQ0096	His-tag affinity SPA beads
Citalopram, [N-Methyl- ³ H]	PerkinElmer	NET1039250UC	
ThermoMixer C	Eppendorf	5382000023	heating block for thermostability assay
ThermoTop	Eppendorf	5308000003	
SmartBlock PCR 96, thermoblock for PCR plates	Eppendorf	5306000006	
0.2 μ m syringe filter	Olympus Plastics	25-243	
1 L filter system	Corning	430517	
2 L flat bottom tissue culture flask	Genemate	F-5909-2000	
2 L baffled tissue culture flask	Genemate	F-5909-2000B	
CO ₂ incubator	Thermo Scientific	3950	
Forma Orbital Shaker	Thermo Scientific	416	
Strep-Tactin resin	Iba Life Sciences	2-1208-025	Strep affinity resin
Extruder	Northern Lipids		
Li-Cor imaging system	Odyssey		western blot imaging system
XK16 column	GE Healthcare	28-9889-37	column used for Strep-Tactin and Talon purificaion
100 kDa MWCO protein concentrator	Millipore	UFC910096	
30 kDa MWCO protein concentrator	Millipore	UFC903024	
Äkta FPLC	GE Healthcare	UPC-900	
HPLC	Shimadzu	51476	
Superose 6 (10/300) column	GE Healthcare	17-5172-01	Used for FSEC
Tangential flow apparatus	Pall Filtron		

0.2 µm filter tangential flow cell	Pall Filtron	PSM20C11	
30 kDa MWCO tangential flow concentrator	Pall Filtron	OS030T12	
Talon resin	Clontech	635504	His-tag affinity resin used for Fab purification
1 mL HiTrap SP column	GE Healthcare	17115101	Cation exchanger used for Fab purification
Superdex 200 10/300 GL column	GE Healthcare	17-5175-01	Used for SEC separation of SERT-8B6
24-well VDXm plate	Hampton Research	HR3-306	
18 mm coverslips	Hampton Research	HR3-239	
Virocyt virus counter	Virocyt	2100	
MicroBeta Trilux	PerkinElmer	1450	96-well scintillation counter
HiTrap SP column	GE Healthcare	17115101	
Sertraline	Sigma	S6319	