

Materials List for:

Purification of Native Complexes for Structural Study Using a Tandem Affinity Tag Method

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Materials

Name	Company	Catalog Number	Comments
S. cerevisiae TAP tagged strain	Open Biosystems	YSC1177	This is the primary yeast strain used to develop the TAP protocol. Its background is S288C: ATCC 201388: MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, SNU71::TAP::HIS3MX6
Coffee grinder	Mr. Coffee	IDS77	Used for cell lysis
Hemocytometer, Bright Line	Hausser Scientific	3120	Used to assess cell lysis
JA 9.100 centrifuge rotor	Beckman Coulter, Inc.		Used to harvest the yeast cells
JA 20 fixed-angle centrifuge rotor	Beckman Coulter, Inc.		Used to clear the cell extract of non-soluble cellular material
Ti 60 fixed-angle centrifuge rotor	Beckman Coulter, Inc.		Used to further clear the soluble cell extract
Thermomixer	Eppendorf	R5355	Temperature controlled shaker
Novex gel system	Thermo Fisher Scientific		
IgG resin	GE Healthcare	17-0969-01	Sepharose 6 fast flow
Calmodulin resin	Agilent Technologies, Inc.	214303	Affinity resin
Protease inhibitor cocktail, mini tablets	Sigma Aldrich	589297	Mini cOmplete ultra EDTA-free tablets
Protease inhibitor cocktail, large tablets	Sigma Aldrich	5892953	cOmplete ultra EDTA-free tablets
Phenylmethanesulfonyl fluoride (PMSF)			Dissolved in isopropanol
2 ml Bio-spin column	Bio-Rad Laboratories, Inc.	7326008	Used to pack and wash the Calmodulin resin
10 ml poly-prep column	Bio-Rad Laboratories, Inc.	7311550	Used to pack and wash the IgG resin
Precast native PAGE Bis-Tris gels	Life Technologies	BN1002	Novex NativePAGE Bis-Tris 4 - 16% precast polyacrylamide gels
NativeMark protein standard	Thermo Fisher Scientific	LC0725	Unstained protein standard used for native PAGE. Load 7.5 μl for a silver stained gel and 5 μl for a SYPRO Ruby stained gel
Precast SDS PAGE Bis-Tris gels	Life Technologies	NP0321	Novex Nu-PAGE Bis-Tris 4 - 12% precast polyacrylamide gels
PageRuler protein standard	Thermo Fisher Scientific	26614	Unstained protein standard used for Western blotting
SDS running buffer	Life Technologies	NP0001	1x NuPAGE MOPS SDS Buffer
TAP antibody	Thermo Fisher Scientific	CAB1001	Primary antibody against CBP tag

Secondary antibody	Thermo Fisher Scientific	31341	Goat anti-rabbit alkaline phosphatase conjugated
BCIP/NBT	Thermo Fisher Scientific	34042	5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium
Dialysis units	Thermo Fisher Scientific	88401	Slide-A-Lyzer mini dialysis units
Centrifugal filter units, 100kDa MWCO	EMD Millipore	UFC5100008	Amicon Ultra-0.5 Centrifugal Filter Unit with Ultracel-100 membrane
Detergent absorbing beads	Bio-Rad Laboratories, Inc.	1523920	Bio-bead SM-2 absorbants
SYBR Green II	Thermo Fisher Scientific	S-7564	Flourescent dye for nucleic acid staining, when detecting with SYPRO Ruby present, use excitation wavelength of 488 nm and emission wavelength of 532 nm
SYPRO Ruby	Molecular Probes	S-12000	Flourescent dye for protein staining, when detecting with SYBR Green II present, use excitation wavelength of 457 nm and emission wavelength of 670 nm
Copper grids	Electron Microscopy Sciences	G400-CP	