

Materials List for:

Budding Yeast Protein Extraction and Purification for the Study of Function, Interactions, and Post-translational Modifications

Eva Paige Szymanski¹, Oliver Kerscher¹

¹Integrated Science Center, Biology Department, The College of William & Mary

Correspondence to: Oliver Kerscher at opkers@wm.edu

URL: <https://www.jove.com/video/50921>

DOI: [doi:10.3791/50921](https://doi.org/10.3791/50921)

Materials

Name	Company	Catalog Number	Comments
Omni Bead Ruptor 24	Omni International	19-010	
Yeast ORF strain in BG1805	ThermoScientific	YSC3869	GAL+ yeast strain that can be used for induction and extraction
pYES2.1 TOPO vector	Life Technologies	K4150-01	GAL+ yeast strain that can be used for induction and extraction; contains a V5/6xHIS tag
Halt Protease Inhibitor Single-Use Cocktail (100x)	Thermo Scientific	1860932	
2.0 ml Skirted Tube with Tethered Screw Cap	BioExpress	C-3369-3	Make sure that the tube properly fits in the bead ruptor
Glass beads, acid-washed, 425-600 µm (30-40 US sieve)	Sigma-Aldrich	G8772	
Corning Costar Spin-X centrifuge tube filters	Sigma-Aldrich	CLS8161	Use for additional filtering of clarified lysate
TALON Metal Affinity Resin	Clontech	635502	For the purification of 6xHIS tagged proteins
NuPAGE LDS Sample Buffer (4x)	Life Technologies	NP0007	To prepare and/or elute samples prior to SDS-PAGE and Western Blotting
NuPAGE 4-12% Bis-Tris Gel	Life Technologies	NP0321BOX	Precast gels often used for SDS-PAGE prior to Western Blotting
Simply Blue	Life Technologies	#LC6060	Protein gel stain
Mammalian Lysis Buffer	Promega	G9381	Alternative commercial lysis buffer
Anti-V5 agarose	Sigma	A7345	Method of immunoprecipitation
ECL	Millipore	WBKL S0 050	
PVDF membrane	Millipore	IPVH00010	
BIS-TRIS gels	Life Technologies	NP0321BOX	
anti-myc antibody	Covance	mms-150R	
secondary antibody	Abcam	ab97040	Goat pAb to mouse IgG (HRP)