

Materials List for:

A Simple Fluorescence-based Reporter Assay to Identify Cellular Components Required for Ricin Toxin A Chain (RTA) Trafficking in Yeast

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Materials

Name	Company	Catalog Number	Comments
Bacterial and yeast strains			
<i>E. coli</i> BL21 DE3(Gold)	Aligent Technologies	230130	
<i>S. cerevisiae</i> BY4742	Euroscarf	Y10000	
<i>S. cerevisiae</i> BY4742 deletion mutants	Dharmacon	YSC1054	whole collection
Name	Company	Catalog Number	Comments
Plasmids used in this protocol			
pET24a ⁽⁺⁾ (Novagen)	Millipore	69772-3	
pET-RTA _(His6)	Becker <i>et al.</i> (2016) ³		
Name	Company	Catalog Number	Comments
Reagents			
Zymolyase 20T	USBio	Z1000.250	lytic enzyme for cell wall removal
LB broth medium	Thermo Scientific	10855021	15 g agar was added for plate production
YNB	Thermo Scientific	DF0335-15-9	
Ammonium sulfate	Sigma-Aldrich	A4418-100G	
Yeast drop-out mix supplements without leucine	Sigma-Aldrich	Y1376-20G	
Agar	Sigma-Aldrich	05040-100G	
D-glucose	Sigma-Aldrich	G8270-100G	
DTT	Sigma-Aldrich	10197777001	
D-raffinose	Sigma-Aldrich	83400-25G	
D-sorbitol	Sigma-Aldrich	S1876-1KG	
D-galactose	Sigma-Aldrich	G0750-10MG	
G418	Thermo Scientific	11811031	
IPTG	Sigma-Aldrich	I6758-1G	
Imidazole	Roth	3899.1	
PAGE ruler prestained	Fermentas	26616	protein ladder used for Western analysis
Name	Company	Catalog Number	Comments
Material for RTA purification, desalting and reader measurements			
Spectrophotometer Ultrospec 2100 pro	Amersham		
Soniprep 150	MSE		old model, other models available

Fluoroskan Ascent	Thermo Scientific	5210470	old model, not available anymore
ÄKTAPurifier	Thermo Scientific	28406266	Product is discontinued and replaced
HisTRAP HP column	GE Healthcare	17-5248-02	
HiTRAP desalting column	GE Healthcare	11-0003-29	
Midisart sterile filter	Sartorius	16534K	0.2 µm pore size
BCA protein assay kit	Pierce	23225	
660 nm assay kit	Thermo Scientific	22660	
96 well plates	Thermo Scientific	260860	
Name	Company	Catalog Number	Comments
Antibodies (optional)			
Anti-Tetra-His	Qiagen	34670	primary antibody; 1:1,000 dilution
Anti-mouse-HRP	Sigma-Aldrich	A9044-2ML	secondary antibody, 1:10,000 dilution