

Materials List for:

Affinity Precipitation of Active Rho-GEFs Using a GST-tagged Mutant Rho Protein (GST-RhoA(G17A)) from Epithelial Cell Lysates

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URL: <https://www.jove.com/video/3932>

DOI: [doi:10.3791/3932](https://doi.org/10.3791/3932)

Materials

Name	Company	Catalog Number	Comments
pGEX-RhoA(G17A)	Addgene	Will be available soon	Generated in the lab of Dr. Keith Burridge (University of North Carolina) (ref ³)
DH5α	Invitrogen	18258-012	Store -80°C
SOC medium	BioShop Canada	SOC001.10	
LB	BioShop Canada	LBL407.1	Keep sterile
Glycerol	BioShop Canada	GLY002.1	
Ampicillin	BioShop Canada	AMP201.25	Store stock at -20°C
Agar	BioShop Canada	AGR001.500	
IPTG (Isopropyl B-D-thiogalactopyranoside)	Calbiochem	420322	Store stock solution at -20°C
Glutathione Sepharose beads	GE Healthcare	17-0756-01	
PBS 10x	Sigma-Aldrich	D1408	
Hepes	Sigma-Aldrich	H4034	
NaCl	BioShop Canada	SOD001.10	
MgCl ₂	Sigma-Aldrich	M-9272	Add just before use
TX-100	Sigma-Aldrich	X100	
DTT	OmniPur, EMD Millipore	3860	Add just before use
PMSF	Sigma-Aldrich	P-7626	Add just before use
Protease Inhibitor 50x	BD Biosciences	51-21426Z	Add just before use
Complete Mini, EDTA-free 10x	Roche Group	11 836 170 001	Add just before use
Laemmli Sample Buffer 2x	Bio-Rad	161-0737	
β-mercapt-ethanol	Sigma-Aldrich	M7154	Add just before use
Coomassie Brilliant Blue	Bio-Rad	R-250	
Acetic Acid	CALEDON	1000-1	
Methanol	CALEDON	6701-7	
Bio-Rad Protein Assay	Bio-Rad	500-0114	
BLUeye ladder	FroggaBio	PM008-0500	
Table 1. Reagents used			
RC-5B centrifuge	Sorvall, Thermo Scientific	SS-34 Rotor	Use at 4°C
Centrifuge	Sorvall, Thermo Scientific	ST-16R	Use at 4°C
Micro centrifuge	Eppendorf	5417R	Use at 4°C
Bacterial shaker	INFORS HT Ecotron	AG CH-1403 Bottmingen	Use at 37°C or at 22°C

Sonicator	Branson	Sonifier-450	Use at RT
Rotator	Glas-Col	099A CR4012	Use at 4°C
Table 2. Specific equipments used			