

Materials List for:

Inducible LAP-tagged Stable Cell Lines for Investigating Protein Function, Spatiotemporal Localization and Protein Interaction Networks

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Materials

Name	Company	Catalog Number	Comments
Flp-In T-REx Core Kit	Invitrogen	K6500-01	Kit for generating cell lines that contain an FRT site and TrtR expression
PETG, 5x	Nunc, Inc.	73520-734	Roller bottle for growing cells
PETG, 2.5x	Nunc, Inc.	73520-420	Roller bottle for growing cells
Cell stackers	Corning CellSTACK	3271	Cell stacker for growing cells
500 ml conical centrifuge tubes	Corning	431123	Tubes for harvesting cells
Anti-GFP antibody	Invitrogen	A11122	Rabbit anti GFP antibody
Affiprep Protein A beads	Biorad	156-0006	Used as a matrix for conjugating anti-GFP antibodies
Dimethylpimelimidate (DMP)	ThermoFisher Scientific	21667	Used for conjugating anti-GFP antibodies to Protein A beads
TLA100.3 tubes	Beckman	349622	Tubes for centrifuging protein lysates during the clearing step
TEV protease	Invitrogen	12575-015	Used for cleaving the GFP tag off of N-terminal LAP-tagged proteins
Precession Protease	GE Healthcare	27-0843-01	Used for cleaving the GFP tag off of C-terminal LAP-tagged proteins
S-protein agarose	Novagen	69704	Used as a second affinity matrix during the purification of LAP-tagged protein complexes
QIAquick DNA gel extraction kit	Qiagen	28704/28706	For use in purifying PCR products from an agarose gel
BP clonase II	Invitrogen	11789020	Used for cloning ORF PCR products into the pDONR221 shuttle vector
LR clonase II	Invitrogen	11791020	Used for cloning the ORF of the gene of interest into the pGLAP1 LAP-tagging vector
<i>ccdB</i> Survival 2 T1 ^R <i>E. coli</i>	Invitrogen	A10460	Used for propagating shuttle vectors and pGLAP empty vectors
Fugene 6	Promega	E2691	Transfection reagent for transfecting vectors into human cells
Tetracycline	Invitrogen	Q100-19	Drug for inducing Dox/Tet inducible protein expression
Doxycycline	Clontech	631311	Drug for inducing Dox/Tet inducible protein expression

Hygromycin B	Invitrogen	10687010	Drug for selecting stable LAP-tagged integrants
Kanamycin	Corning	61-176-RG	Drug for selecting Kanamycin resistant bacterial colonies
Ampicillin	Fisher	BP1760-5	Drug for selecting Ampicillin resistant bacterial colonies
4-20% Tris Glycine SDS-PAGE gels	Biorad	4561094	Used for separating protein samples and final LAP-tag purification eluates
Silver Stain Plus Kit	Biorad	1610449	Used for silver staining the eluates of LAP-tagged purifications and samples collected throughout the purification process
Coomassie Blue stain	Invitrogen	LC6060	Used for staining SDS-PAGE gels to visualize LAP-tagged purifications and cutting out protein bands, mass spectrometry compatible
Shuttle vector pDONR221	Invitrogen	12536017	Shuttle vector for cloning the ORFs of genes of interest
Flippase expressing vector pOG44	Invitrogen	V600520	Vector that expresses the Flippase recombinase for integrating LAP-tagged genes into the genome of FRT site containing cell lines
Platinum Taq DNA Polymerase	ThermoFisher Scientific	10966018	Used for PCR amplification of the ORFs of genes of interest
4x Laemmli sample buffer	Biorad	1610747	Sample buffer for eluting purified LAP-tagged protein complexes from the bead matrix
Luria broth (LB) media	Fisher	BP9723-2	Used for growing DH5 α bacteria
DNA miniprep kit	Promega	A1222	Used for making DNA plasmid minipreps
DMEM/F12 media	Hyclone	SH30023.01	For growing Hek293 human cells
FBS lacking Tet	Altanta Biologicals	S10350	Used for making -Tet DMEM/F12 media for generating and growing inducible LAP-tagged stable cell lines
Trypsin	Hyclone	SH30042.01	For lifting Hek293 cell foci from plates
Protease inhibitor tablets	Roche	11836170001	Used for making protocol buffers, EDTA-free
10% nonyl phenoxyethoxyethanol	Roche	11332473001	Used for making protocol buffers
PBS	Corning	21-040-CM	Used for making protocol buffers
Tween-20	Fisher	BP337-500	Used for making protocol buffers
Sodium Borate	Fisher	S249-500	Used for making protocol buffers
Boric Acid	Fisher	A78-500	Used for making protocol buffers
Ethanolamine	Calbiochem	34115	Used for making protocol buffers
NaCl	Fisher	P217-3	Used for making protocol buffers
KCl	Fisher	BP358-10	Used for making protocol buffers
Dithiothreitol (DTT)	Fisher	BP172-25	Used for making protocol buffers
MgCl ₂	Fisher	M33-500	Used for making protocol buffers
Tris base	Fisher	BP152-5	Used for making protocol buffers