

Materials List for:

# Identification of Kinase-substrate Pairs Using High Throughput Screening

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## Materials

Name	Company	Catalog Number	Comments
Lysis buffer	Made in house		See Protocol step 1.1
10x kinase buffer	Made in house		See Protocol step 1.2
10x M-ATP	Made in house		See Protocol step 1.3
Human kinase plasmids	Orfeome, Invitrogen, Origene		GST-tagged in house
96 well plates	Fisher Scientific	CS003595	
293T cells	ATCC	CRL-11268	
DMEM	Fisher Scientific	SH3002201	supplement with 100 U/ml penicillin, 100 µg/ml streptomycin, 10% fetal calf serum.
CO <sub>2</sub> incubator	Sanyo	MCO-17AIC	
15 cm cell culture dishes	Fisher Scientific	877224	
Reduced serum medium	Invitrogen	22600-050	
Lipid-based transfection reagent	Invitrogen	11668-019	
Automated liquid dispenser	Thermo Scientific	5840300	
Small cassette attachment	Thermo Scientific	24073295	
Standard cassette attachment	Thermo Scientific	14072670	
4 mM pervanadate	Made in house		See Protocol step 3.1
0.25 M CaCl <sub>2</sub>	Made in house		
Multichannel pipette (20-200 µl)	Labnet	p4812-200	
Multichannel pipette (1-10 µl)	Thermo Scientific	4661040	
V-bottom 6-well plates	Evergreen Scientific	290-8116-01V	
Glutathione coated 96-well plates	Fisher Scientific	PI-15240	
Hybridization oven	Biostad	350355	
GST tagged substrate	Made in house		
Myelin Basic Protein (MBP)	Sigma	M1891	
Repeater pipette (1 ml)	Eppendorf	22266209	
<sup>32</sup> P gamma-ATP	Perkin Elmer	BLU502Z500UC	
2x SDS lysis buffer (100 ml)	Made in house		See Protocol step 1.4
26-well precast TGX gels	BioRad	567-1045	gel percentage required is dependent on the molecular weight of the substrate of interest
Coomassie stain	Made in house		0.1% Coomassie R250, 10% acetic acid, 40% methanol
Coomassie destain	Made in house		10% acetic acid, 20% methanol

Labeled gel containers	Made in house		Used plastic lids from empty tip boxes, just big enough to contain one gel
Whatman filter paper	Fisher Scientific	57144	
Cellophane sheets (2)	BioRad	165-0963	
Gel dryer	Labconco	4330150	
Double emulsion autoradiography film	VWR	IB1651454	
Film cassette	Fisher Scientific	FBAC-1417	
Intensifying screen	Fisher Scientific	FBIS-1417	
Plate sealing rubber roller	Sigma	R1275	