

Materials List for:**Utilizing a Comprehensive Immunoprecipitation Enrichment System to Identify an Endogenous Post-translational Modification Profile for Target Proteins**Henrick Horita¹, Andy Law¹, Kim Middleton¹¹R&D Department, Cytoskeleton Inc.Correspondence to: Henrick Horita at henrickh@cytoskeleton.comURL: <https://www.jove.com/video/56912>DOI: [doi:10.3791/56912](https://doi.org/10.3791/56912)**Materials**

Name	Company	Catalog Number	Comments
Cell lysis/genomic DNA removal and analysis			
1x phosphate buffered saline (PBS)	common buffer		Recipe: NaCl, KCl, Na ₂ HPO ₄ , KH ₂ PO ₄
BlastR lysis buffer	Cytoskeleton Inc.	BLST01	Proprietary mixture of detergents, salts, and other buffer additives
Radioimmunoprecipitation assay buffer (RIPA)	common buffer		Recipe: Tris-HCl, NaCl, SDS, Sodium Deoxycholate, Igepal
Mammalian protein extraction reagent (mPER)	Thermofisher	78503	Proprietary mixture of detergents, salts, and other buffer additives
Immunoprecipitation (IP) Lysis	Pierce	87787	Proprietary mixture of detergents, salts, and other buffer additives
1% sodium dodecyl sulfate (SDS) denaturing buffer	common buffer		Recipe: PBS, SDS, EDTA, EGTA
Laemmli	common buffer		Recipe: Tris-HCl, SDS, glycerol, bromophenol blue
BlastR dilution buffer	Cytoskeleton Inc.	BDB01	Proprietary mixture of detergents, salts, and other buffer additives
Protease Inhibitor Cocktail	Cytoskeleton Inc.	PIC02	
N-Ethylmaleimide (NEM) & N,N,N',N'-Tetrakis(2-pyridylmethyl)ethylenediamine (TPEN)	Cytoskeleton Inc.	NEM09BB	
Trichostatin A (TSA) & Nicotinamide	Cytoskeleton Inc.	TSA01	
Activated Sodium Orthovanadate (Na ₃ VO ₄)	Cytoskeleton Inc.	PYI01	
cell scrapers	Costar	3008	
BlastR Filter	Cytoskeleton Inc.	BLR02	
BD Insulin syringe needle	BD	08290-3284-11	
sonicator (Branson Sonifier)	Branson	Sonifier 450	
Precision Red Advanced Protein Reagent	Cytoskeleton Inc.	ADV02	
1 mL cuvettes	Fisher	14955127	
Spectrophotometer (Genesys20)	Thermofisher	4001	Other spectrophotometers can be used to measure protein concentration
Agarose	Fisher	BP1356-500	
1kb Deoxyribonucleic acid (DNA) ladder	NEB	N3232L	

DNA gel box	Thermofisher	7312 B1A	
Tris/Borate/EDTA (TBE) buffer	common buffer		Recipe: Tris-HCl, Boric Acid, EDTA
PTM Immunoprecipitation			
Phosphotyrosine beads	Cytoskeleton Inc.	APY03-beads	
Ubiquitination beads	Cytoskeleton Inc.	UBA01-beads	
SUMOylation 2/3 beads	Cytoskeleton Inc.	ASM24-beads	
Acetylation beads	Cytoskeleton Inc.	AAC04-beads	
Phosphotyrosine control beads	Cytoskeleton Inc.	CIG01-beads	
Ubiquitination control beads	Cytoskeleton Inc.	CUB02-beads	
SUMOylation 2/3 control beads	Cytoskeleton Inc.	CIG01-beads	
Acetylation control beads	Cytoskeleton Inc.	CIG02-beads	
BlastR wash buffer	Cytoskeleton Inc.	BWB02	Proprietary mixture of detergents, salts, and other buffer additives
Bead elution buffer	Cytoskeleton Inc.	BEB01	Proprietary mixture of detergents, salts, and other buffer additives
microcentrifuge	Eppendorf	5417	Other microcentrifuges can be used
tube rotator	ATR	RKVSD	Other end-over-end tube rotators can be used
protein loading tips	VWR	37001-150	
spin columns	Cytoskeleton Inc.	SPN22	
heat block 95 °C	Benchmark	BSH1002	
SDS-PAGE/Transfer/Western analysis			
5x sample buffer	common buffer		recipe: Bromophenol blue, dithiothreitol (DTT), Glycerol, SDS, Tris-HCl
novex wedge well 4-20% Tris-gly gels	Invitrogen	XP04200BOX	The large wedgewells are excellent for loading large volumes
1x Running buffer	common buffer		recipe: Tris-HCl , Glycine, SDS
seeblue protein ladder	Life Technologies	LC5625	
electrophoresis cell	Invitrogen	Xcell surelock electrophoresis cell	Other electrophoresis cells can be used, however these are compatible the novex wedgewell gels
power supply	Biorad	PowerPac HC	Other power supplies can be used
1x Transfer buffer	common buffer		recipe: Tris-HCl, glycine, methanol
Transfer cell	Biorad	mini trans-blot cell	Other transfer cells can be used
Immobilon- P membranes (PVDF)	millipore	IPVH 304F0	
non-fat milk	thrive	813503015095	
Tris buffered saline with tween (TBST)	common buffer		recipe: Tris-HCl, NaCl, Tween 20
Chemiluminescent Detection Reagent	Cytoskeleton Inc.	CLRA/CLRB	
Cell growth and treatment			
Dulbecco's Modified Eagle's medium	ATCC	30-2002	
Fetal bovine serum	ATLAS	F-0500-A	
Epidermal growth factor	Cytoskeleton Inc.	CN02-A	
150 mm cell culture plates	Corning	430599	