

Materials List for

# Mass Spectrometry Analysis to Identify Ubiquitylation of EYFP-tagged CENP-A (EYFP-CENP-A)

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## Materials

Name	Company	Catalog Number	Comments
<b>Equipments/Tools</b>			
0.5 ml protein low binding tubes	Eppendorf	022431064	For mass spectrometry analysis
10cm cell culture dish	BIOFIL/JET, China	700224	10 cm tissue culture dish (Yohei lab, PN63)
6 Well Cell Culture Cluster	Fisher/Corning Incorporated	07-200-83	6-well culture plate
CentriVap	LABCONCO	-	Benchtop vacuum concentrator for vacuum dry peptides for mass spectrometry analysis
ChromXP C18CL, 120A, 15 cm x 75 μm	Eksigent Technologies	805-00120	Liquid chromatography (RPLC) column for mass spectrometry analysis
HCX PL APO 100x oil immersion lens	Leica	LEICA HCX PL APO NA 1.40 OIL PHE	100X Oil immersion lens
HCX PL APO 63x oil immersion lens	Leica	LEICA HCX PL APO NA 1.40 OIL PH 3 CS	63X Oil immersion lens
Immobilon-FL PVDF Transfer Membrane	EMD Millipore	IPVH00010	For western blot
Leica DM IRE2 motorized fluorescence microscope	Leica	-	motorized fluorescence microscope
Leica EL6000 compact light source	Leica		External light source for fluorescent excitation
Micro Cover glass (22 mm x 22 mm)	Surgipath	105	Cover glass (22 mm x 22 mm)
Model V16-2 polyacrylamide gel electrophoresis apparatus	Apogee Electrophoresis/CORE Life Sciences	31071010	Gel electrophoresis apparatus I to apply bigger SDS-PAGE gel
nanoLC.2D	Eksigent Technologies	-	liquid chromatography system for mass spectrometry analysis
NuPAGE 4%-12% Bis-Tris Protein Gels	Thermo Fisher	NP0335BOX	The commercially available 4%-12% Bis-Tris protein gels for mass spectrometry analysis

Olympus FLUOVIEW FV3000 confocal laser scanning microscope	Olympus	-	Confocal laser scanning microscope ( <a href="https://www.olympus-lifescience.com.cn/en/support/downloads/#!dOpen=%23detail847250519">https://www.olympus-lifescience.com.cn/en/support/downloads/#!dOpen=%23detail847250519</a> )
ORCA-R2 Digital CCD camera	Hamamatsu	C10600-10B	CCD camera
PAP Pen	Binding Site	AD100.1	For a water repellent barrier in immunofluorescent staining
TISSUE CULTURE DISHES 10CM	VWR	25382-166	10 cm tissue culture dish
Vertical electrophoresis for gel running (big size)	Junyi, China	JY-SCZ6+	Gel electrophoresis apparatus II to apply bigger SDS-PAGE gel (Yohei lab, PE23)
VWR Micro Slides, Frosted	VWR International	48312-013	Micro slides
<b>Primary antibodies</b>			
Anti-CENP-A antibody	Stressgen/Enzo Life Sciences	KAM-CC006	Mouse monoclonal antibody
Anti-CENP-B antibody	Novus Biologicals	H00001059-B01P	Mouse monoclonal antibody
anti-GAPDH	ABCAM	ab37168	Rabbit polyclonal antibody
anti-GAPDH	Invitrogen	PA1987	Rabbit polyclonal antibody
anti-GFP antibody	ANTI #76 (Homemade antibody)		Rabbit polyclonal antibody
anti-HA (3F10)	Roche	11815016001	Rat monoclonal antibody
anti-HJURP	Proteintech Group	15283-1-AP	Rabbit polyclonal antibody
anti-Ubiquitin	Bethyl Laboratories	A300-317A-1	Rabbit polyclonal antibody
<b>Reagents</b>			
Bio-Rad Protein Assay	Bio-Rad	500-0006	Commercial protein assay reagent I for measurement of protein concentration (compatible with 0.1% SDS)
Branson SONIFIER 450			Sonicator
Branson Ultrasonics sonicator Microtip Step, Solid, Threaded 9.5 mm	VWR Scientific Products Inc.	33995-325	Disruptor horn for sonication
Branson Ultrasonics sonicator Microtip Tapered 6.5 mm	VWR Scientific Products Inc.	33996-185	Microtip for sonication
Buffer A1	-	-	20 mM Tris-HCl, pH 7.4; 50 mM NaCl; 0.5% Nonidet P-40; 0.5% deoxycholate; 0.5% SDS; 1 mM EDTA; complete EDTA-free protease inhibitor reagent
Complete EDTA-free protease inhibitor cocktail	Roche	11-873-580-001	Complete EDTA-free protease inhibitor reagent for buffer A1
Coomassie brilliant blue R-250	BBI Life Sciences	CAS 6104-59-2	Coomassie blue solution for mass spectrometry analysis
Crystal violet solution (2.3% crystal violet, 0.1% ammonium oxalate, 20% ethanol)	Sigma-Aldrich	HT90132-1L	For colony staining
DAPI	SIGMA-SLDRICH	D9542	For nuclear staining
DMEM: F12 Medium	ATCC	30-2006	DMEM: F12 Medium
Fetal Bovine Serum, certified, heat inactivated, US origin	Life Technologies/Gibco	10082	FBS (fetal bovine serum)
High-glucose DMEM (Dulbecco's modified Eagle's medium)	Life Technologies/BioWhittaker	12-604	high-glucose DMEM
Lipofectamin 3000	Life Technologies/Invitrogen	L3000	Transfection reagent I for chemical transfection
Lipofectamin 3000, P3000 solution	Life Technologies/Invitrogen	L3000	Transfection reagent II for chemical transfection
Methanol	Fisher	A412-4	Fixation reagent

Non fat powdered milk (approved substitution for carnation powdered milk)	Fisher Scientific	NC9255871 (Reorder No. 190915; Lot# 90629)	Non-fat skim milk
Opti-MEM I	Life Technologies/Invitrogen	31985	Reduced serum media
p-phenylenediamine	SIGMA-SLDRICH	P6001	For mounting medium
Penicillin, Streptomycin; Liquid	Fisher/Gibco	15-140	Penicillin-streptomycin
Poly-L-Lysine SOLUTION	SIGMA-SLDRICH	P 8920	Poly-L-Lysine, 0.1% w/v, in water
Polyethyleneimine [PEI]; 1.0 mg/ml	Polysciences	23966-2	Transfection reagent III for chemical transfection
Protein A sepharose CL-4B beads	GE Healthcare/Amersham	17-0963-03	Protein A sepharose CL-4B beads for in vivo ubiquitylation assays using pQCXIP-EYFP-CENP-A
Restore Western Blot Stripping Buffer	Thermo Scientific	PI21059	Western Blot Stripping Buffer I
Sequencing grade trypsin	Promega	V5111	For mass spectrometry analysis
SuperSignal West Femto Maximum Sensitivity Substrate	Thermo	34095	Ultra-sensitive enhanced chemiluminescent (ECL) substrate
UltraPure Distilled Water	Life Technologies/Invitrogen/Gibco	10977	Sterile tissue culture grade water
Western Blot Stripping Buffer II ((50 mM Tris-HCl, pH 6.85; 2% SDS; 50 mM DTT; 100 mM 2-Mercaptoethanol)	-	-	Western Blot Stripping Buffer II
<b>Secondary antibodies</b>			
Alexa Fluor 488 Goat Anti-Rabbit IgG	Life Technologies/Invitrogen	A11008	fluorophore-conjugated secondary antibody (Affinity-purified secondary antibody)
Alexa Fluor 594 Goat Anti-Mouse IgG	Life Technologies/Invitrogen	A11005	fluorophore-conjugated secondary antibody (Affinity-purified secondary antibody)
<b>Softwares</b>			
Acquisition FV31S-SW software	Olympus	-	Software C1 ( <a href="https://www.olympus-lifescience.com.cn/en/support/downloads/#!dlOpen=%23detail847250519">https://www.olympus-lifescience.com.cn/en/support/downloads/#!dlOpen=%23detail847250519</a> )
Analysis FV31S-DT software	Olympus	-	Software C2 ( <a href="https://www.olympus-lifescience.com.cn/en/support/downloads/#!dlOpen=%23detail847250519">https://www.olympus-lifescience.com.cn/en/support/downloads/#!dlOpen=%23detail847250519</a> )
cellSens Dimension software Ver. 1.18	Olympus	-	Software C3 ( <a href="https://www.olympus-lifescience.com.cn/en/software/cellsens/">https://www.olympus-lifescience.com.cn/en/software/cellsens/</a> )
Image Studio Analysis Software Ver 4.0	LI-COR Biosciences	-	Software D
Molecular Imager Versadoc MP4000 System	Bio-Rad	-	Chemiluminescence imager for immunoblot detection
Odyssey CLx Infrared imaging System	LI-COR Biosciences	-	Infrared imaging system for immunoblot detection
OpenCFU software	-	-	For colony counting ( <a href="http://openclu.sourceforge.net/">http://openclu.sourceforge.net/</a> )
Openlab version 5.5.2. Scientific Imaging Software	Improvison/PerkinElmer	-	Software A
ProteinPilot Software version 4.5	AB SCIEX	-	Software F for mass spectrometry analysis
Quantity One 1-D analysis software	Bio-Rad	-	Software E
TripleTOF 5600+ System	AB SCIEX	-	Mass spectrometry instrument
Volocity version 6.3 3D Image Analysis Software (Volocity Acquisition)	PerkinElmer	-	Software B1

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Volocity version 6.3 3D Image Analysis Software (Volocity Quantification)	PerkinElmer	-	Software B2
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